SOIL SCIENCE

VOLUME 47

JANUARY–JUNE, 1939

RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY
U. S. A.

PUBLISHED BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MARYLAND

SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

ADVISORY STAFF

A. W. BLAIR, J. S. JOFFE, L. L. LEE, H. C. MCLEAN, A. L. PRINCE, J. W. SHIVE, H. B. SPRAGUE, R. L. STARKEY, AND S. A. WAKSMAN, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

CONSULTING EDITORS

F. J. ALWAY
University of Minnesota, St. Paul, Minn.
K. ASO
Imperial University, Tokyo, Japan
C. BARTHEL
Lantbrukshögskolan, Uppsala, Sweden
RICHARD BRADFIELD
College of Agriculture, Cornell University, Ithaca, N. Y.
B. C. BURT
Imperial Council of Agricultural Research, New Delhi, India
H. J. CONN
New York State Experiment Station, Geneva, N. Y.
ALBERT DEMOLON
Ministry of Agriculture, Paris, France
E. B. FRED
University of Wisconsin, Madison, Wis.
V. V. GEMMERLING
Agronomitsheskii Institute, Moskovskii Universitat, Moscow, U. S. S. R.
J. E. GREAVES
Utah Agricultural College, Logan, Utah
D. J. HISSINK
Soil Science Institute, Groningen, Holland
D. R. HOAGLAND
University of California, Berkeley, Calif.
O. LEMMERMANN
Berlin-Zehlendorf, Germany
C. B. LIPMAN
University of California, Berkeley, Calif.
BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md.
HENRIK LUNDEGÅRDH
Centralanstalten för jordbruksförsök, Stockholm, Sweden
M. M. MCCOOL
Boyce-Thompson Institute, Yonkers, N. Y.
W. H. MACINTIRE
University of Tennessee, Knoxville, Tenn.
S. MATTSON
Lantbrukshögskolan, Uppsala, Sweden
S. MIKLASEWSKI
Institute of Soil Science, Warsaw, Poland
E. A. MITSCHERLICH
University of Königsberg, Germany
C. A. MOOERS
University of Tennessee, Knoxville, Tenn.
D. N. PRIANISHNIKOV
Timiryazev Academy, Moscow, U. S. S. R.
THEO. REMY
Bonn-Rhein, Germany
G. W. ROBINSON
University College of North Wales, Bangor, Wales
E. J. RUSSELL
Rothamsted Experimental Station, Harpenden, England
O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.
ALEXIUS A. J. DE'SIGMOND
Royal Hungarian Joseph University of Technical Sciences, Budapest, Hungary
N. M. TULAIKOV
Agricultural Experiment Station, Saratov, U. S. S. R.
EMILE H. DEL VILLAR
Forestry Institute, Madrid, Spain
S. WINOGRADSKY
Pasteur Institute, Paris, France

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879

Copyright, 1939, The Williams & Wilkins Company

Science Pub.

CONTENTS

The Mechanism of Cation Exchange in the Montmorillonite-Beidellite-Nontronite Type of Clay Minerals. J. E. GIESEKING 1

The Nature, Extent, and Distribution of Fertilizer Residues in the Soil of Some Old Fertility Plats. W. H. METZGER 15

Chemical Nature of Organic Matter in Differently Cropped Arid Soils. KENNETH R. STEVENS 27

A Microchemical Study of the Effects of Boron Deficiency in Cotton Seedlings. CECIL H. WADLEIGH AND JOHN W. SHIVE 33

Thermophilic Actinomycetes and Fungi in Soils and in Composts. SELMAN A. WAKSMAN, W. W. UMBREIT, AND T. C. CORDON 37

Root Nodule Bacteria of Some Tropical Leguminous Plants: II. Cross-Inoculation Tests within the Cowpea Group. O. N. ALLEN AND ETHEL K. ALLEN 63

Physical Characteristics of Soils: II. Expressing Mechanical Analysis and State of Aggregation of Soils by Single Values. AMAR NATH PURI AND B. R. PURI 77

Influence of Temperature upon the Microbiological Population and Decomposition Processes in Composts of Stable Manure. SELMAN A. WAKSMAN, T. C. CORDON AND N. HULPOI 83

Effect of Phosphates on Nitrifying Capacity of Soils. G. S. FRAPS AND A. J. STERGES 115

The Stimulating Effects of Silicates on Plant Yields in Relation to Anion Displacement. S. J. TOTH 123

The Role of Potassium in Plants: I. Effect of Varying Amounts of Nitrogenous, Carbohydrate, and Mineral Metabolism in the Tomato Plant. MONROE E. WALL 143

Book Reviews 163

Giacomo Rossi, 1872–1938 173

Comparative Study of the Colloids of a Cecil and a Susquehanna Soil Profile. W. P. KELLEY, A. O. WOODFORD, W. H. DORE, AND S. M. BROWN 175

Desert Subsoil Temperatures. WILLIAM V. TURNAGE 195

The Influence of Cropping on the Nitrogen-Fixing Powers of Soil. J. E. GREAVES AND A. F. BRACKEN 201

A Bacterium Antagonistic to *Rhizoctonia solani*. T. C. CORDON AND C. M. HAENSELER 207

Thermophilic Decomposition of Plant Residues in Composts by Pure and Mixed Cultures of Microorganisms. SELMAN A. WAKSMAN AND T. C. CORDON 217

Relative Absorption of Nutrients by Weeds of Arable Land. B. N. SINGH AND L. B. SINGH 227

The Zinc Content of Weeds and Volunteer Grasses and Planted Land Covers. L. H. ROGERS, O. E. GALL, AND R. M. BARNETTE 237

Base Exchange in Soils: I. A Critical Examination of the Methods of Finding Base-Exchange Capacity of Soils. AMAR NATH PURI AND H. L. UPPAL 245

Book Review 255

Cation Interchange between Plant Roots and Soil Colloids. H. JENNY AND R. OVERSTREET 257

Base Content of Corn Plants as Influenced by pH of Substrate and Form of Nitrogen Supply. C. H. WADLEIGH AND J. W. SHIVE 273

Is Sunlight a Factor in Nitrogen Transformation in Soil? B. N. SINGH AND K. M. NAIR 285

Host Plant Specificity among the Medicago in Association with Root-Nodule Bacteria. J. C. BURTON AND P. W. WILSON 293

The Availability, to Crop Plants, of Different Forms of Selenium in the Soil. O. E. Olson and A. L. Moxon 305
Some Moisture Relationships of Soils from Burned and Unburned Longleaf Pine Forests. Frank Heyward 313
A New Species of Azotobacter. Robert L. Starkey and P. K. De 329
Edmund Cecil Shorey, 1865–1939 345
Jacob Goodale Lipman, 1874–1939 ..... facing 346
Capillary Tension as a Measure of the Pore Space Unoccupied by Water in Some Dense Orchard Subsoils. Damon Boynton 347
Preservation of Small Core Soil Samples. R. W. Gerdel 353
Influence of Exchangeable Cations on the Availability of Phosphate in Soils. S. Ravikovitch 357
Effect of Dilution on the Water-Soluble and Exchangeable Bases of Alkali Soils and Its Bearing on the Salt Tolerance of Plants. W. P. Kelley 367
Effect of Organic Matter on the Water-holding Capacity and the Wilting Point of Mineral Soils. George J. Bouyoucos 377
Some Factors Influencing the Heat of Wetting of Soils. H. A. Wadsworth 385
The Control of Nut Grass with Chloropicrin. G. H. Godfrey 391
Some Critical Studies of the Phenoldisulfonic Acid Method for the Determination of Nitrates. Emery M. Roller and Nelson McKaig, Jr. 397
Comparison of Methods of Determining Small Quantities of HCN. O. H. Coleman and Robert Gardner 409
Physical Characteristics of Soils: III. Heat of Wetting. Amar Nath Puri and R. C. Hoon 415
Effect of Certain Mineral Elements on Some Microbiological Activities in Muck Soils. L. M. Turk 425
Effect of Saline Water on Mediterranean Loess Soils. M. Puffeles 447
Plant Growth and the Breakdown of Inorganic Soil Colloids. W. A. Albrecht, Ellis R. Graham, and Carl E. Ferguson 455
Studies on the Nitrogen, Phosphorus, and Mineral Requirements of Alfalfa. A. W. Blair and A. L. Prince 459
Rates of Temperature Changes in Soils at Various Moisture Contents. H. A. Wadsworth 467
Effect of Temperature upon the Moisture-Content—Surface-Force Curve of Soil. H. A. Wadsworth 473
Studies in Electrodialysis of Soils: IV. Effect of Temperature, pH Value, and Degree of Alkalization. Amar Nath Puri, R. C. Hoon and C. L. Dhawan 479
Index 487

ILLUSTRATIONS

PLATES

The Mechanism of Cation Exchange in the Montmorillonite-Beidellite-Nontronite Type of Clay Minerals

Plate 1. X-ray diffraction Diagrams of Montmorillonite to Which Have Been Added Various Amounts of Tributyl Mono-Heptyl Ammonium Iodide 13

Thermophilic Actinomycetes and Fungi in Soils and in Composts

Plate 1. Thermophilic Fungi in Composts of Horse Manure 55
Fig. 1. *Thermomyces* in 50°C. compost, 18 hours old 55
2. *Thermomyces* mycelium in young compost at 50° 55
3. Actively sporulating *Thermomyces* in 50° compost, 48 hours old 55
4. Details of mycelium and spore formation by *Thermomyces*, developing in 48-hour compost 55
5. Rapid destruction of fungus mycelium by bacteria in 6-day-old compost 55
6. Masses of fungus spores, bacteria, actinomycetes spores on remnants of plant material in 15 days 55
Plate 2. Thermophilic Species of Actinomyces in Composts of Horse Manure 57
Fig. 7. *Act. thermophilus* in 50°C. compost, 48 hours old 57
8. *Act. thermophilus* accompanied by bacteria in 65°C. compost, 48 hours old 57
9. *Act. thermofuscus* in 50°C. compost, 19 days old 57
10. *Actinomyces* III in 65°C. compost, 10 days old 57
11. *Actinomyces* III in 65°C. compost, 10 days old 57
12. *Actinomyces* III in 50°C. compost, 15 days old, accompanying fungus mycelium 57
Plate 3. Thermophilic Species of Micromonospora in Composts of Horse Manure 59
Fig. 13. *Micr. vulgaris* in 65°C. compost, 15 days old, showing spores close to mycelium 59
14. *Micr. vulgaris* on short side branches, together with sporulating Thermomyces in 50°C. compost, 2 days old 59
15. *Micr. vulgaris*, showing extensive spore formation on short side branches, in 50°C. compost, 2 days old 59
16. *Micr. chalceae* in 65°C. compost, 6 days old 59
17. *Micr. fusca* in 50°C. compost, 1 day old 59
18. *Micr. fusca* in 50°C. compost, 15 days old 59
Plate 4. Pure Cultures of Actinomycetes 61
Fig. 19. *Act. thermophilus*. Gram stain of a 6-hour slide culture (50°C.) showing germinating spores and initial mycelium development 61
20. *Act. thermophilus*. Aerial mycelium just emerging from substratum mycelium but no spore formation evident. Unstained, living material 61
21. *Act. thermophilus*. Unstained living material at incipient stage of spore formation. Tips of hyphae showing indentation 61
22. *Act. thermophilus*. Unstained living preparation, 10 days old, on Czapek's agar 61

23. *Act. thermofuscus.* Stained with methylene blue, showing spirals and formation of spores 61
24. *Micr. vulgaris.* Gram stain of an 18-hour slide culture (60°C.), showing germinating spores and initial mycelium formation 61
25. *Micr. vulgaris.* Early stages of spore formation. Gram stain. Showing swelling of branches and incipient spores 61
26. *Micr. vulgaris.* Later stages of spore formation 61

INFLUENCE OF TEMPERATURE UPON THE MICROBIOLOGICAL POPULATION AND DECOMPOSITION PROCESSES IN COMPOSTS OF STABLE MANURE

Plate 1. Microbiological Population active in the Decomposition of Horse Manure at 28°C 101
Fig. 1–3. One-day-old compost, showing different types of bacteria, nonspore-forming and spore-forming, singly, in chains, and in colonies 101
4–5. Two-day-old compost, showing predominance of heavy rods 101
6. Four-day-old compost, showing heterogeneous bacterial population and protozoan cysts 101
7. Nine-day-old compost, showing large masses of bacteria and filaments of actinomycetes 101
8. Thirty-four-day-old compost, showing different types of bacteria distributed singly and in colonies 101
Plate 2. Other Members of the Microbiological Population of Composts Kept at 28°C. . 103
Fig. 9. Fifteen-day-old compost. Fungus filaments and protozoan cysts 103
10. Fifteen-day-old compost. Fungus filaments and perithecea-like bodies 103
11. Nineteen-day-old compost. Fungus mycelium with single cylindrical-shaped cells, borne on side branches 103
12. Thirty-four-day-old compost. *Cephalothecium*-like organisms 103
13. Thirty-four-day-old compost. Mycelium and sexual spores of *Zygorhynchus* 103
14. Ciliates found in 34- and 40-day-old composts 103
15. Ciliates found in 34- and 40-day-old composts 103
16. Nematodes in 15-day-old compost 103
Plate 3. Microbiological Population Active in the Decomposition of Horse Manure at 50°C 105
Fig. 17. One-day-old compost, showing chains and masses of spore-forming bacteria 105
18–19. One-day-old compost, showing extensive development of two types of actinomycetes, namely *Actinomyces* and *Micromonospora* .. 105
20. Two-day-old compost. Extensive bacterial development accompanied by fungus spores 105
21. Two-day-old compost, showing spherical bodies, accompanying rod-shaped bacteria 105
22. Seven-day-old compost, showing actinomycetes, spherical bodies and protozoan cysts (?) 105
23. Seven-day-old compost, showing attack upon plant fiber by bacteria followed by protozoa (?) 105
24. Extensive development of *Thermomyces* in 15-day-old compost 105
Plate 4. Microbiological Population in Composts of Horse Manure at 50°C 107
Fig. 25. Nineteen-day-old compost, showing *Oidium*-like fungus and bacterial spores 107

26. Nineteen-day-old compost, showing chains of bacteria accompanying fungus mycelium 107
27. Twenty-two-day-old compost, showing development of *Actinomyces thermophilus* 107
28. Thirty-four-day-old compost, showing *Actinomyces thermofuscus* and *Oidium*-like fungus 107
29. Forty-day-old compost, showing bacterial colonies, individual bacterial cells, and bacterial spores 107
30. Four-day-old compost, showing spores of fungi, filaments of actinomycetes, *Micromonospora* spores, and coccoid cells 107
31. Seven-day-old compost showing spores of fungi (?), bacterial cells, and *Micromonospora* spores 107
32. Forty-day-old compost, showing large numbers of bacterial spores, protozoan cysts, and filaments of actinomycetes 107

Plate 5. Microbiological Population of Composts of Horse Manure Kept at 65°C 109
Fig. 33. Twenty-four-hour-old compost, showing long rod-shaped, spore-forming bacteria 109
34. Two-day-old compost, showing chains of spore-forming bacteria and more lightly stained masses of bacterial spores 109
35. Two-day-old compost, showing short and broad bacteria, thin rod-shaped bacteria, and masses of spores 109
36. Two-day-old compost, showing abundance of *Micromonospora vulgaris*. 109
37–38. Four-day-old compost, showing masses of *Micromonospora fusca*.. 109
39. Five-day-old compost, showing a new type of *Micromonospora* related to *M. chalceae* 109
40. Seven-day-old compost, showing *Micromonospora chalceae* 109

Plate 6. Microbiological Population of Composts of Horse Manure Kept at 65°C 111
Fig. 41. Nineteen-day-old compost, showing fragmented mycelium and spores of actinomycetes 111
42. Nine-day-old compost, showing masses of spores of *Micromonospora*.. 111
43. Fifteen-day-old compost, showing *Actinomyces thermofuscus* 111
44. Nineteen-day-old compost, showing *Micromonospora vulgaris* 111
45. Twenty-two-day-old compost, showing *Micromonospora chalceae* 111
46. Twenty-two-day-old compost, showing another preparation of *Micromonospora chalceae* 111
47. Thirty-four-day-old compost, showing masses of *Micromonospora* spores and larger bodies, of an unknown nature 111
48. Forty-day-old compost, showing masses of bacteria, various bacterial spores, and *Micromonospora* spores 111

Plate 7. Microbiological Population of Horse Manure Kept at 75°C 113
Fig. 49. Twenty-four-hour-old compost, showing several types of rod-shaped bacteria 113
50. Four-day-old compost, showing two distinct types of bacteria, short curved rods and long chain-forming rods 113
51. Five-day-old compost, showing predominance of long, spore-forming rods of the *Plectridium* type, with a number of short rods 113
52. Fifteen-day-old compost, showing granulation of long rods 113
53. Fifteen-day-old compost, showing abundance of bacterial spores and few vegetative cells 113
54. Another 15-day-old compost preparation, showing heavy rods and *Micromonospora* mycelium 113
55. Forty-day-old preparation, showing mycelium and spores of *Micromonospora vulgaris* 113

THE STIMULATING EFFECTS OF SILICATES ON PLANT YIELDS IN RELATION TO ANION DISPLACEMENT

Plate 1. Effect of Lime, Magnesium Silicate, and Calcium Silicate, with and without Phosphate, on Yields of Sudan Grass 141
Fig. 1. Effect of lime and silicate on yields of sudan grass 141
2. Effect of lime and of magnesium and calcium silicates with rock phosphate on yields of sudan grass 141
Giacomo Rossi ..... Frontispiece

A BACTERIUM ANTAGONISTIC TO RHIZOCTONIA SOLANI

Plate 1. Antagonistic Effect of *Bacillus simplex* on *Rhizoctonia solani* 215
Fig. 1a. *Rhizoctonia* alone. 1b. *Rhizoctonia* with agar blocks from the zone near a bacterial colony. 1c. *Rhizoctonia* with one agar block and one bacterial colony. 1d. *Rhizoctonia* with bacterial colonies 215
2. Higher magnification of *Rhizoctonia* and agar block taken from the sterile zone near a bacterial colony .....
3. Normal *Rhizoctonia* hyphae 215
4. *Rhizoctonia* hyphae in the proximity of the bacterium 215

THERMOPHILIC DECOMPOSITION OF PLANT RESIDUES IN COMPOSTS BY PURE AND MIXED CULTURES OF MICROORGANISMS

Plate 1. Growth of *Psalliota campestris* on Thermophilic Compost of Plant Residues .. 225

HOST PLANT SPECIFICITY AMONG THE MEDICAGO IN ASSOCIATION WITH ROOT-NODULE BACTERIA

Plate 1. Host Plant Specificity in Species and Varieties of Medicago 303
Fig. 1. Growth of three species of *Medicago* on nitrogen-poor sand when inoculated with *Rh. meliloti*, strains 100, 105, and 110 303
2. Roots of *M. arabica* at harvest, showing that invasion of the plant had occurred even though no nitrogen fixation resulted except with strain 105 303
3. Growth of *M. hispida* and three varieties of *M. sativa* (Ladak, Hairy Peruvian, Grimm) on nitrogen-poor sand when inoculated with *Rh. meliloti* 100, 105, and 110 303

SOME MOISTURE RELATIONSHIPS OF SOILS FROM BURNED AND UNBURNED LONGLEAF PINE FORESTS

Plate 1. Fig. 1. Typical open-grown stand of second-growth longleaf pine 325
2. Forest floor under closed stand of second-growth longleaf pine 325
Plate 2. Fig. 1. Dense ground cover typical of long-unburned areas 327
2. Typical view of annually burned areas, photographed approximately 2 months after a fire 327

A NEW SPECIES OF AZOTOBACTER

Plate 1. Fig. 1. Colony development of *Az. indicum* upon silica gel plate containing sucrose and seeded with granules of soils 341
2. Development of *Az. indicum* on streaked plate of nitrogen-free sucrose agar 341
3. Colonies of *Az. indicum* on glucose agar plate 341
Plate 2. Fig. 4. Cells of *Az. indicum* stained with phenolic rose-bengal 343
5. Unstained cells of *Az. indicum* 343
Edmund Cecil Shorey 344

PRESERVATION OF SMALL CORE SOIL SAMPLES

Plate 1. Soil Core Samples Preserved with Bakelite; One Face Ground and Polished to Show Structure 355
Fig. 1. Core samples from fallow ground showing development of an erosion pavement by late winter and spring rains 355
2. Variations in type of structure 355

THE CONTROL OF NUT GRASS WITH CHLOROPICRIN

Plate 1. Effect of Chloropicrin Fumigation on Nut Grass 395
Fig. 1. Nut grass from experiment 1 395
2. The chloropicrin-treated bed, about 1 month after treatment 395

TEXT-FIGURES

THE MECHANISM OF CATION EXCHANGE IN THE MONTMORILLONITE-BEIDELLITE-NONTRONITE TYPE OF CLAY MINERALS

Fig. 1. Crystal Structure of Montmorillonite Showing the Variable (001) Spacing Along the c Axis 2
2. Broken Bonds Resulting from the Breaking of a Silicon-Oxygen Plane of a Clay Crystal 2
3. Effect of Various Amounts of Adsorbed Tributyl Mono-Heptyl Ammonium ions on the (001) Spacings of Montmorillonite 9
4. Hydrogen Exchanged from a H-Montmorillonite System by Addition of Increasing Amounts of Tributyl Mono-Heptyl Ammonium Iodide 9

THE NATURE, EXTENT, AND DISTRIBUTION OF FERTILIZER RESIDUES IN THE SOIL OF SOME OLD FERTILITY PLATS

Fig. 1. Effect of Soil Treatments on the Easily Soluble Phosphorus of the Soil at Various Depths 17

PHYSICAL CHARACTERISTICS OF SOILS: II. EXPRESSING MECHANICAL ANALYSIS AND STATE OF AGGREGATION OF SOILS BY SINGLE VALUES

Fig. 1. Example of a Summation Curve 78
2. Summation Curves of an Alluvial Soil (P.C. 1) and of a Chernozem Soil (P.C. 2) .. 80
3. Summation Curves of Soils to Illustrate a Low and a High Value of σ 80

INFLUENCE OF TEMPERATURE UPON THE MICROBIOLOGICAL POPULATION AND DECOMPOSITION PROCESSES IN COMPOSTS OF STABLE MANURE

Fig. 1. Influence of Temperature (°C.) upon the Course of Decomposition of Horse Manure, as Measured by Loss of Total Dry Material and Carbohydrates 92
2. Chemical Changes in Horse Manure Decomposing at Different Temperatures (°C.), on Percentage Basis 93

THE ROLE OF POTASSIUM IN PLANTS: I. EFFECT OF VARYING AMOUNTS OF POTASSIUM ON NITROGENOUS, CARBOHYDRATE, AND MINERAL METABOLISM IN THE TOMATO PLANT

Fig. 1. Nitrogenous and Carbohydrate Fractions of Tomato Plants Grown with Nutrient Solutions Containing Different Amounts of Potassium 150
2. Mineral Fractions of Tomato Plants Grown with Nutrient Solutions Containing Different Amounts of Potassium 151

COMPARATIVE STUDY OF THE COLLOIDS OF A CECIL AND A SUSQUEHANNA SOIL PROFILE

Fig. 1. Dehydration Curves of Cecil Colloids 180
2. Dehydration Curves of Susquehanna Colloids 181
3. The Sequence of Atomic Planes of Kaolinite 191
4. The Sequence of Atomic Planes of Montmorillonite 192

DESERT SUBSOIL TEMPERATURES

Fig. 1. Thermometer and Thermocouple Records in Soil at a Depth of 6 Feet 196
2. Soil Temperatures, as Determined by Thermocouples, at Depths of 3, 6, and 12 Feet 197

BASE EXCHANGE IN SOILS: I. A CRITICAL EXAMINATION OF THE METHODS OF FINDING BASE-EXCHANGE CAPACITY OF SOILS

Fig. 1. Titration Curves of Soils with the Quinhydrone and the Glass Electrode 246

CATION INTERCHANGE BETWEEN PLANT ROOTS AND SOIL COLLOIDS

Fig. 1. Schematic Representation of Base Exchange or Ionic Exchange 258
2. Schematic Representation of Contact Exchange 258
3. Schematic Representation of Contact Intake 259
4. Schematic Representation of Contact Depletion 259

HOST PLANT SPECIFICITY AMONG THE MEDICAGO IN ASSOCIATION WITH ROOT-NODULE BACTERIA

Fig. 1. Effect of Strain of *Rh. meliloti* on Nitrogen Fixation by Varieties of *Medicago sativum* 294
2. Effect of Strain of *Rh. meliloti* on Nitrogen Fixation by Species of Medicago 300

THE AVAILABILITY, TO CROP PLANTS, OF DIFFERENT FORMS OF SELENIUM IN THE SOIL

Fig. 1. Members of the Pierre Formation 306
2. The Selenium Cycle in Nature 306

SOME MOISTURE RELATIONSHIPS OF SOILS FROM BURNED AND UNBURNED LONGLEAF PINE FORESTS

Fig. 1. Soil Moisture Percentages of Burned and Unburned Plots in Terms of Difference Between Means in Relation to Standard Error of Difference, with Significance of Results Indicated 318

CAPILLARY TENSION AS A MEASURE OF THE PORE SPACE UNOCCUPIED BY WATER IN SOME DENSE ORCHARD SUBSOILS

Fig. 1. Capillary Tension, Soil Moisture, and Pore Space Occupied by Gas—Location 1, Cornell University Orchard 348
2. Capillary Tension, Soil Moisture, and Pore Space Occupied by Gas—Location 2, Cornell University Orchard 349

INFLUENCE OF EXCHANGEABLE CATIONS ON THE AVAILABILITY OF PHOSPHATE IN SOILS

Fig. 1. Liberation of Adsorbed PO_4 as Influenced by Various Exchangeable Cations 361
2. Adsorption of PO_4 by Soils with Various Exchangeable Cations 363

PHYSICAL CHARACTERISTICS OF SOILS: III. HEAT OF WETTING

Fig. 1. Relation Between Relative Humidity and Moisture Percentage of Soils 417
2. Relation Between Heat of Wetting and Relative Humidity of Soil 417

3. Relation Between Relative Humidity and Moisture Percentage of Soils Containing Different Single Bases 420
4. Relation Between Relative Humidity and Heat of Wetting of Soils Containing Different Single Bases 420
5. Heat of Wetting of Soil in Different Liquids 421

EFFECT OF CERTAIN MINERAL ELEMENTS ON SOME MICROBIOLOGICAL ACTIVITIES IN MUCK SOILS

Fig. 1. Carbon Dioxide Production in Crane Muck 440

RATES OF TEMPERATURE CHANGES IN SOILS AT VARIOUS MOISTURE CONTENTS

Fig. 1. Rate of Temperature Change for Inert Materials Initially Cold 469
2. Rate of Temperature Change for Soils 469
3. Rate of Change of Temperature of Moist Soils 470
4. Rate of Change of Temperature of Superior Clay Loam at 8 Per cent Moisture ... 470

EFFECT OF TEMPERATURE UPON THE MOISTURE-CONTENT—SURFACE-FORCE CURVE OF SOIL

Fig. 1. Relative-Humidity—Moisture-Content Curves of Soils at Various Temperatures 476

STUDIES IN ELECTRODIALYSIS OF SOILS: IV. EFFECT OF TEMPERATURE, pH VALUE, AND DEGREE OF ALKALIZATION

Fig. 1. Effect of pH on the Rate of Recovery of Bases by Electrodialysis 480
2. Relation Between Degree of Alkalization and Ca/Na Ratio in the Electrodialyzate and Yield of Wheat 484
3. Effect of Temperature on the Speed of Electrodialysis 484

THE MECHANISM OF CATION EXCHANGE IN THE MONTMORILLONITE-BEIDELLITE-NONTRONITE TYPE[1] OF CLAY MINERALS[2]

J. E. GIESEKING[3]

Illinois Agricultural Experiment Station

Received for publication June 17, 1938

Cation exchange in clays has long been considered as a surface phenomenon of the finer clay particles. Kelley and Jenny (13) have expressed the belief that the cation exchange power of the natural bentonites is due to exposed OH groups of the crystal lattice. In 1933 Hofmann, Endell, and Wilm (10) reported that bentonite and related clays, upon the adsorption of water and other polar compounds, exhibit a reversible, one dimensional, inner crystalline swelling, resulting in a variable spacing between the (001) planes of the montmorillonite crystals. Hofmann and his associates have pictured the crystal structure of montmorillonite and the variable spacing within which water may be adsorbed as shown in figure 1. Nontronite, according to Gruner (7), also gives (001) spacings which vary with the water content of its crystals. Other substances reported to exhibit this same type of swelling are: graphite oxide, reported by Hofmann and Frenzel (12), and basic cobalt sulfate, reported by Feitknecht and Fischer (5).

Hofmann and Bilke (8) found the maximum swelling, or variation in the (001) spacing, of Na-montmorillonite to be much greater than that of H- and Ca-montmorillonite. This is cited as evidence that a portion of the exchangeable cations are held within the variable spacing of the crystal lattice. In addition to this, they suggest that broken bonds resulting from broken lattices (fig. 2) play a rôle in cation exchange. In another paper Hofmann, Endell, and Bilke (9) express the opinion that the exchangeable bases are not held within the variable spacing but are held entirely on the outer surface of the montmorillonite crystal.

According to Marshall (15) negative charges within the variable spacing arise from the partial substitutions of magnesium (two positive charges) for aluminum (three positive charges) in montmorillonite, and from the partial substitution of aluminum (three positive charges) for silicon (four positive

[1] In this discussion, those minerals having a variable (001) spacing with varying water contents are considered as belonging to this group.

[2] Contribution from the division of soil physics, department of agronomy, University of Illinois, Urbana, Illinois. Published with the approval of the director of the experiment station.

[3] Associate in soil physics.

charges) in beidellite. Larsen and Steiger (14) have shown the existence of a complete isomorphous series of minerals formed by various degrees of substitution of ferric iron for aluminum in beidellite. In this series $Al_2O_3 \cdot 3SiO_2 \cdot nH_2O$ corresponds to beidellite at one end of the series, and $Fe_2O_3 \cdot 3SiO_2 \cdot nH_2O$ corresponds to nontronite at the other end. Since iron and aluminum have the same valence, no change in the charge on the lattice should result from this substitution.

Correns and Mehmel (4) obtained different values for the index of refraction of montmorillonite when different liquids were used for immersion. In working with minerals from soil clays, Van Baren (1) found that immersion liquids containing organic amino compounds gave high indexes of refraction.

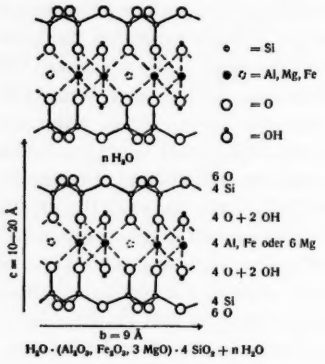


FIG. 1

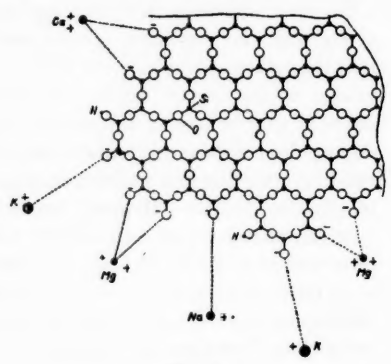


FIG. 2

FIG. 1. CRYSTAL STRUCTURE OF MONTMORILLONITE SHOWING THE VARIABLE (001) SPACING ALONG THE C AXIS

Reproduced from Hofmann and Bilke (8)

FIG. 2. BROKEN BONDS RESULTING FROM THE BREAKING OF A SILICON-OXYGEN PLANE OF A CLAY CRYSTAL

Reproduced from Hofmann and Bilke (8)

During the investigation of various cations in base exchange reactions with Putnam clay, Gieseking and Jenny (6) found methylene blue to be very effective in replacing adsorbed cations. These results suggested the possibility of using large substituted ammonium ions of the NH_3R^+, $NH_2R_2^+$, NHR_3^+, and NR_4^+ types to throw more light on the mechanism of cation exchange in the montmorillonite-beidellite-nontronite type of clays. If exchange takes place within the variable spacing of these clays the large cations should give greater (001) spacings than the common cations when adsorbed by the clay.

EXPERIMENTAL TECHNIC

The clays used in this study were Wyoming bentonite, nontronite from the Woody district in California, Hartsburg clay, and Cisne clay. The last two

clays were extracted from the corresponding soil types occurring in Illinois. In each case $<1\mu$ fractions, dispersed in distilled water, were used. The Illinois clays were found to contain relatively small quantities of the montmorillonite-beidellite-nontronite type of clay. Fractionation[4] of these soil clays without the use of a dispersing agent failed to show a concentration of the montmorillonite-beidellite-nontronite type of clay in the finest fraction. The H-montmorillonite used in this investigation was prepared from a mixture of coarse, fine, and superfine fractions of Wyoming bentonite. These fractions have been defined by Bray, Grim, and Kerr (3). The adsorbed cations were removed by electrodialysis, and the capacity (94 m.e. per 100 gm.) was determined by leaching a sample with normal barium chloride and titrating the replaced hydrogen.

The dispersed clays were treated with water solutions of the hydrochlorides or hydroiodides of the various amines used. The clays were found to be completely flocculated after small quantities of the salts of the amines were added to the suspensions. The treated suspensions were shaken several hours in a mechanical shaker and filtered. The flocculated clays were washed with distilled water, air dried, and ground in an agate mortar. A powder x-ray diffraction pattern was taken of each treated sample, FeKα radiation being used. The diffracted radiation was recorded on a flat film at a distance of 5 cm. from the sample for spacings of less than 20 Å. and at a distance of 10 cm. for spacings of more than 20 Å.

EXPERIMENTAL RESULTS

The (001) spacings for the original clay crystals, together with the corresponding values for a series of clays after saturation with large substituted ammonium ions, are reported in table 1. These values also represent the length of the c axis of the unit cell of the clay crystal.

The results of another series of measurements obtained on samples of H-montmorillonite to which had been added varying amounts of tributyl mono-heptyl ammonium iodide,[5] are plotted in figures 3 and 4. Figure 3 shows the (001) spacings, and figure 4 shows the amount of hydrogen replaced in each case. The x-ray diffraction diagrams of this series of samples are reproduced in plate 1.

DISCUSSION OF RESULTS

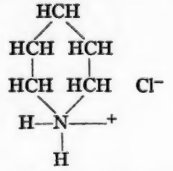
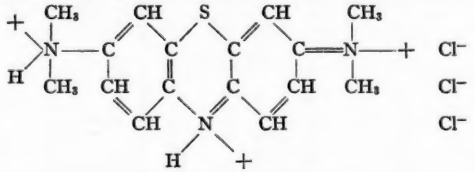
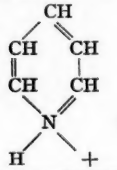
The data show that the replacement of calcium and hydrogen ions by large substituted ammonium ions gives rise to greater (001) spacings in the montmorillonite-beidellite-nontronite type of minerals. Furthermore, the intensity of the diffracted radiation from the (001) planes, as shown in plate 1, increases with an increase in the amount of complex ion added, which suggests an

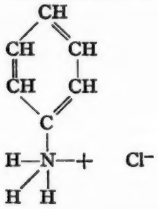
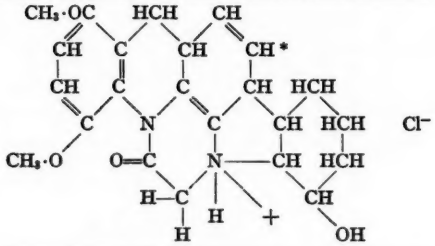
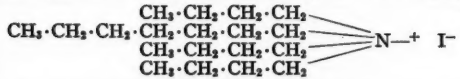
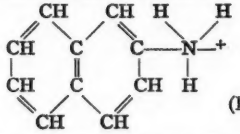
[4] Fractionations by W. L. Nelson and L. E. Ensminger.

[5] The author is indebted to C. S. Marvel, of the chemistry department, for the tributyl mono-heptyl ammonium iodide used in this investigation.

TABLE 1

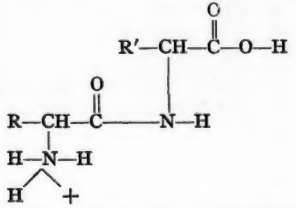
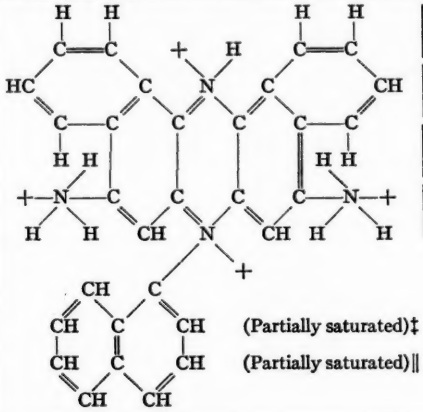
Distance between the (001) planes of montmorillonite, nontronite, Hartsburg clay, and Cisne clay before and after saturation with large substituted ammonium ions

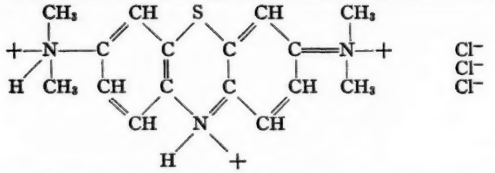
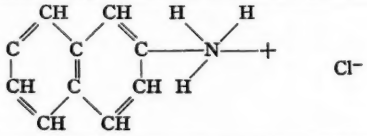
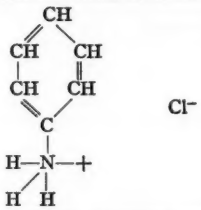
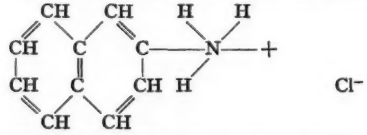
AMINE OR CATION	STRUCTURAL FORMULA OF CATION	(001) SPACINGS			
		Dried and exposed over $Mg(ClO_4)_2$	Dried at 105°C.	Air dried	Under water
		Å.	Å.	Å.	Å.
Wyoming Bentonite (Montmorillonite)					
Hydrogen	H^+	12.5		13.7	
Calcium	Ca^{++}			12.0	
Piperidine				13.5	
Methylene blue				14.0	
Pyridine			14.2	14.7	

Aniline			14.8	15.0	15.2
Brucine			16.5	16.8	16.7
Tributyl mono-heptyl ammonium iodide		17.3	18.0	17.2	18.2
β-Naphthylamine	 (Partially saturated)		17.7	18.2 16.2	18.0

* Structure proposed by Perkin and Robinson.

TABLE 1—(*Continued*)

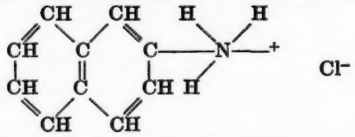
AMINE OR CATION	STRUCTURAL FORMULA OF CATION	(001) SPACINGS			
		Dried and exposed over $Mg(ClO_4)_2$	Dried at 105°C.	Air dried	Under water
		Å.	Å.	Å.	Å.
Wyoming Bentonite (Montmorillonite)—Concluded					
Gelatine			27.0	27.6	
Magdala red	 (Saturated)†		29.0	30.0	30.0
	(Partially saturated)‡			24.2	
	(Partially saturated)‖			17.5	
Nontronite					
Calcium	Ca^{++}			15.5	

		Nontronite			
Calcium	Ca^{++}			15.5	
Methylene blue				16.4§	
β-Naphthylamine				18.2	
		Harlsburg Clay			
Calcium	Ca^{++}			14.5	
Aniline				15.0	
β-Naphthylamine				17.5	

†‡‖ This series was prepared by adding magdala red stepwise to a bentonite suspension: ‖ the least amount of magdala red, ‡ the medium amount, and † the highest amount.

§ This value was determined by L. E. Ensminger.

TABLE 1—(*Concluded*)

AMINE OR CATION	STRUCTURAL FORMULA OF CATION	(001) SPACINGS			
		Dried and exposed over $Mg(ClO_4)_2$	Dried at 105°C.	Air dried	Under water
		Å.	Å.	Å.	Å.
Cisne Clay					
Calcium	Ca^{++}			15.0¶	
β-Naphthylamine	 Cl^-			16.7¶	

¶ These values determined by W. L. Nelson.

increase in the density of the diffracting plane. This indicates that a portion of the cations are being adsorbed within the variable spacing.

A comparison of the structural formulas and the (001) spacings obtained for the clays treated with the various amines shows that the spacing obtained is not always dependent on the dimensions of the cation in the free state. For example, the large brucine cation gives a smaller spacing than does β-napthyl-

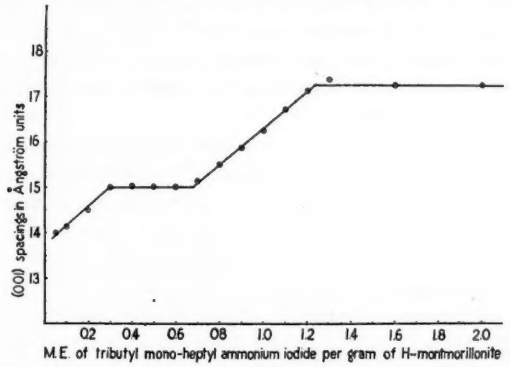


FIG. 3. EFFECT OF VARIOUS AMOUNTS OF ADSORBED TRIBUTYL MONO-HEPTYL AMMONIUM IONS ON THE (001) SPACINGS OF MONTMORILLONITE

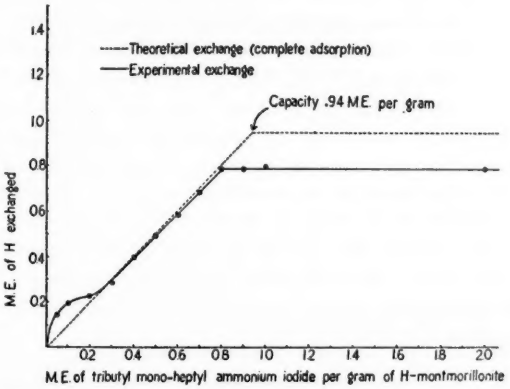


FIG. 4. HYDROGEN EXCHANGED FROM A H-MONTMORILLONITE SYSTEM BY ADDITION OF INCREASING AMOUNTS OF TRIBUTYL MONO-HEPTYL AMMONIUM IODIDE

amine, the smaller of the two cations. In this connection the spacing resulting from the absorption of a given cation between two planes should depend upon the activity and the effective dimension in a direction perpendicular to the two planes. The latter in turn will depend on hydration, orientation, and tendency toward packing, deformation, and polarization under pressure.

According to Hofmann and associates (8, 9, 10, 11, 12) and Bradley, Grim,

and Clark (2) montmorillonite exhibits (001) spacings the magnitude of which is dependent upon the water content. Calcium, sodium, or hydrogen systems were used by these investigators. The data in table 1 show that the (001) spacings of montmorillonite saturated with various large substituted ammonium ions were not significantly affected by variations in water content of the sample. These treated samples no longer showed the swelling and dispersion in water so characteristic of Ca-, Na-, or H-montmorillonite. In order to avoid any effect of adsorbed water on the (001) spacings of the samples partially saturated with tributyl mono-heptyl ammonium iodide, these samples were dried and exposed over anhydrous magnesium perchlorate, the values obtained being plotted in figure 3.

The results plotted in figures 3 and 4 indicate that tributyl mono-heptyl ammonium iodide is very completely adsorbed by montmorillonite below the saturation point for this cation. Attempts to replace these large cations by hydrogen have shown only a trace to be removed after several treatments with normal hydrochloric acid. These adsorbed cations, however, are readily replaced by other cations of equal size.

Since these cations are so strongly adsorbed and the effect of adsorbed water on the (001) spacings of the treated clay mineral is negligible, the activity and hydration of the cation may be disregarded. It seems, therefore, that orientation of the adsorbed cation or its tendency toward packing, deformation, or polarization must be used to explain the results plotted in figure 3. Cations adsorbed within the variable spacing, as has been pictured, will be subjected to pressure. When only a few of these large cations are present, they may not be able to exhibit their preferred orientation and shape. As more and more of the cations are adsorbed they will be more nearly able to assume preferred states, because of less pressure per cation. The flat part of the curve in figure 3 at 15 Å. may be the result of more efficient packing of the cations before another layer starts forming above 15 Å. Instead of another layer's forming above 15 Å., there is the possibility that a different type of orientation is becoming effective, giving the same trend as that shown by the lower part of the curve. Since the slope of the curve above the flat portion is virtually the same as that below, it seems probable that the same forces were acting in both cases.

CONCLUSIONS

Minerals of the montmorillonite-beidellite-montronite type adsorb large substituted ammonium ions, giving rise to (001) spacings greater than those of the same minerals saturated with smaller cations such as calcium or hydrogen. The diffracted radiation from the (001) planes of these minerals, treated with the complex cations, was much more intense than the same diffraction from the calcium and hydrogen systems. The gradual increase in the amount of complex cation added to montmorillonite systems results in increases in (001) spacings and intensity of diffraction from these planes until a maximum is

reached. These results have been interpreted as showing that a portion of the complex cations were adsorbed within the variable (001) spacings of the minerals.

The large complex cations used in this investigation were very strongly adsorbed by montmorillonite. They were found to be exchanged by other cations of approximately the same size, but they were not exchanged by hydrogen, which is very effective in replacing small cations.

The substituted ammonium cations were found to be very effective in flocculating the dispersed clays.

Montmorillonite saturated with the large substituted ammonium ions did not show the water adsorption, swelling, and dispersion characteristic of Ca-, Na-, and H-montmorillonite. The (001) spacings of the complex ammonium systems did not vary with the water content of the system.

REFERENCES

(1) Baren, F. A. van 1936 Über den Einfluss vershiedener Flüssigkeiten auf den Brechungsindex von Tonmineralien. *Ztschr. Krist.* 95: 464–469.

(2) Bradley, W. F., Grim, R. E., and Clark, G. L. 1937 A study of the behavior of montmorillonite upon wetting. *Ztschr. Krist.* (A) 97: 216–222.

(3) Bray, R. H., Grim, R. E., and Kerr, P. F. 1935 Application of clay mineral technique to Illinois clay and shale. *Bul. Geol. Soc. Amer.* 46 (12): 1909–1926.

(4) Correns, C. W., and Mehmel, M. 1936 Über den optischen und röntgenographischen Nachweis von Kaolinit, Halloysit, und Montmorillonit. *Ztschr. Krist.* 94: 337–348.

(5) Feitknecht, W., and Fischer, G. 1935 Zur Chemie und Morphologie der basischen Salze zweiwertiger Metalle. *Helvetica Chim. Acta* 18: 40–60.

(6) Gieseking, J. E., and Jenny, H. 1936 Behavior of polyvalent cations in base exchange. *Soil Sci.* 42: 273–280.

(7) Gruner, J. W. 1935 The structural relationship of nontronite and montmorillonite. *Amer. Mineralogist* 20: 475–483.

(8) Hofmann, U., and Bilke, W. 1936 Über die innerkristalline Quellung und das Basenaustauschvermögen des Montmorillonits. *Kolloid Ztschr.*, 77: 238–251.

(9) Hofmann, U., Endell, K., and Bilke, W. 1935 Die Quellung von Bentonit und seine technische Anwendung. *Ztschr. Elektrochem.* 41: 469–471.

(10) Hofmann, U., Endell, K., and Wilm, D. 1933 Kristallstruktur und Quellung von Montmorillonit. *Ztschr. Krist.* 86: 340–348.

(11) Hofmann, U., Endell, K., and Wilm, D. 1934 Röntgenographische und kolloidchemische Untersuchungen über Ton. *Angew. Chem.* 47: 539–547.

(12) Hofmann, U., and Frenzel, A. 1930 Quellung von Graphit und die Bildung von Graphitsäure. *Ber. Deut. Chem. Gesell.* (B) 63: 1248–1262.

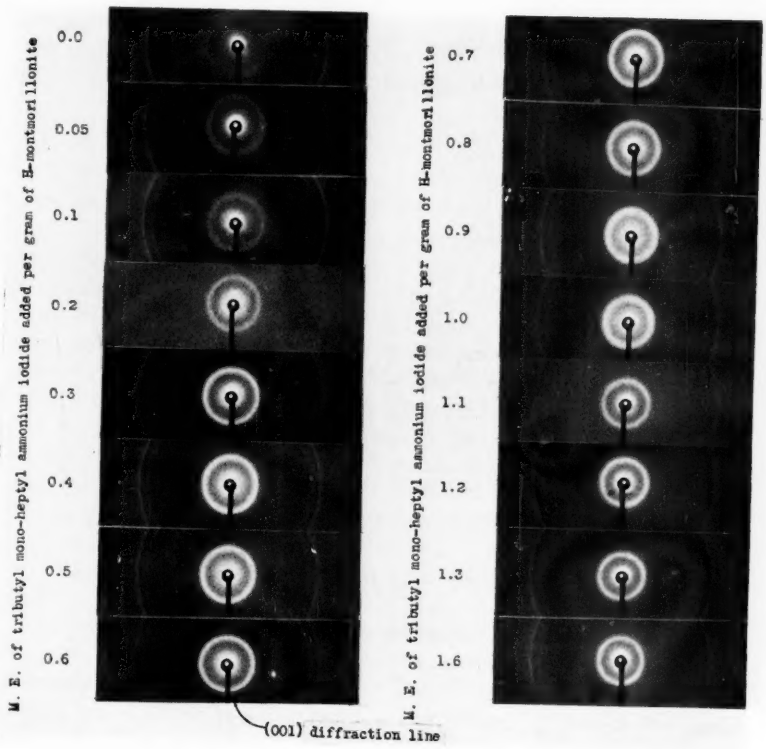
(13) Kelley, W. P., and Jenny, H. 1936 The relation of crystal structure to base exchange and its bearing on base exchange in soils. *Soil Sci.* 41: 367–382.

(14) Larsen, E. S., and Steiger, G. 1928 Dehydration and optical studies of alunogen, nontronite, and griffithite. *Amer. Jour. Sci.* 215: 1–19.

(15) Marshall, C. E. 1935 The importance of the lattice structure of the clays for the study of soils. *Jour. Soc. Chem. Indus.* 54: 393–398.

PLATE 1

X-RAY DIFFRACTION DIAGRAMS OF MONTMORILLONITE TO WHICH HAVE BEEN ADDED VARIOUS AMOUNTS OF TRIBUTYL MONO-HEPTYL AMMONIUM IODIDE


M. E. of tributyl mono-heptyl ammonium iodide added per gram of H-montmorillonite
0.0
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.6
(001) diffraction line

THE NATURE, EXTENT, AND DISTRIBUTION OF FERTILIZER RESIDUES IN THE SOIL OF SOME OLD FERTILITY PLATS[1]

W. H. METZGER[2]

Kansas Agricultural Experiment Station

Received for publication July 2, 1938

In a continuous alfalfa experiment on the agronomy farm of the Kansas Agricultural Experiment Station, fertilizer treatments were applied annually for 27 years. All fertilizer, manure, and lime applications were made as top-dressings to an established stand of alfalfa. The land was plowed only three times during these years—in 1910 after only one top-dressing of fertilizer had been made, in 1923, and in 1928—each time for the purpose of reëstablishing the stand of alfalfa. In each case, plowing was shallow, the maximum depth being about 4 or 5 inches. No other tillage operations were practiced which would aid in the incorporation of the fertilizer, manure, or lime with the soil. The alfalfa was regularly cut, and all top growth was removed as hay.

In 1936 the experiment was discontinued, and the soil was sampled in each plat at the following depths, in inches: 0–4, 4–6, 6–9, 9–12, 12–18, 18–24, 24–36. These samples, in whole or in part, were analyzed for total phosphorus, easily soluble phosphorus (Truog method), total potassium, exchangeable potassium, exchangeable sodium, pH values, and base exchange capacity of the original soil and of the electrodialyzed soil.

The amounts of the various fertilizing materials applied are indicated in table 1.

The precipitation at Manhattan has averaged 31.49 inches over a period of 79 years. About three fourths of this occurs during the 6 months April to September, inclusive. Because of the high rate of evaporation prevailing in this area and the constant removal of moisture by the growing alfalfa plants, leaching in the plats on which this work was based was necessarily restricted. This fact must be considered when the data are studied. The soil is a prairie soil, classified as Derby silt loam. It is derived from the weathering of wind deposits. Because of the removal of considerable surface soil by erosion before the experiment was started the dark brown A horizon is rather shallow. The B horizon is reddish brown silty clay loam. Its moderately well developed fragmental structure renders it moderately friable. The

[1] Contribution No. 281 from the department of agronomy, Kansas Agricultural Experiment Station.

[2] Associate professor of soils, Kansas State College.

soil contains many iron-bearing minerals in its finer fractions, particularly in the B horizon, which give it relatively high phosphorus fixing capacity. The pH values of both A and B horizons fall within the limits 5.5 to 5.8.

EASILY SOLUBLE PHOSPHORUS AND ITS DISTRIBUTION IN THE SOIL

In 1934 the author presented (2) the results of a study of easily soluble phosphorus in the soil of these plats. The samples analyzed at that time were taken in 1923 after 14 annual applications of fertilizers had been made.

In the studies here reported the phosphorus was extracted and determined by the Truog procedure (7). The results are listed in table 2 and are shown graphically in figure 1. The accumulated easily soluble phosphorus from the treatments was determined by plotting the phosphorus values for the control plats 2, 5, 8, 11, connecting the points by straight lines, reading off

TABLE 1

Rates of fertilizer application

FERTILIZER	ANNUAL APPLICATION	TOTAL PHOSPHORUS (P) APPLIED IN 27 YEARS	TOTAL POTASSIUM (K) APPLIED IN 27 YEARS
	lbs./A.	*lbs./A.*	*lbs./A.*
Superphosphate	190	356	
Rock phosphate	380	1,395	
Sulfate of potash	180*		
	90†		1,226
Nitrate of soda	240		
Manure (plats 7, 9, 12)	5,000	300	1,260
(plat 10)	10,000	600	2,520
Lime (hydrated)	1,000‡		

* For 6 years.
† For 21 years.
‡ As needed.

the corresponding values for the treated plats, and comparing these with the values obtained by analysis. Plats 1 and 12, both treated plats, were compared directly with control plats 2 and 11, respectively.

It is evident that the accumulation of easily soluble phosphorus was largest in plat 7, which was treated with manure and rock phosphate. This plat, however, received 1,395 pounds of phosphorus in rock phosphate and 300 pounds in manure, a total of 1,695 pounds of phosphorus, in the 27 years, or about 4½ times as much phosphorus as was applied on each of the superphosphate-treated plats. Easily soluble phosphorus accumulated at all depths in plat 7 except in the 24–36-inch layer.

There appears to have been some accumulation at all depths in the superphosphate plat, though the amount at each of the two lowest depths is so small that it might be accounted for by natural soil variability. No accumulation below 12 inches is apparent in the plat treated with superphosphate plus

TABLE 2

Easily soluble phosphorus at various depths under old alfalfa sod

Expressed as parts per million of air-dry soil

DEPTH	PLAT 1* SUPER-PHOS-PHATE	PLAT 2 CHECK	PLAT 3 SUPER-PHOS-PHATE, K_2SO_4	PLAT 4 K_2SO_4	PLAT 5 CHECK	PLAT 6 SUPER-PHOS-PHATE, K_2SO_4, $NaNO_3$	PLAT 7 MANURE 2½ T., ROCK PHOS-PHATE	PLAT 8 CHECK	PLAT 9 MANURE 2½ T.	PLAT 10 MANURE 5 T.	PLAT 11 CHECK	PLAT 12 MANURE 2½ T., LIME
inches												
0–4	40.0	9.3	25.0	4.0	8.0	40.0	195.0	9.9	15.1	23.5	9.8	20.8
4–6	44.3	9.5	20.1	2.5	4.5	13.0	152.2	4.7	4.3	9.0	6.5	9.0
6–9	21.7	8.7	11.8	1.4	2.2	6.6	105.8	3.5	5.2	5.8	2.9	5.4
9–12	14.0	5.9	9.8	1.3	2.5	4.2	23.6	3.7	3.9	5.3	2.9	5.5
12–18	11.7	5.2	4.6	1.5	1.7	3.8	14.1	5.4	2.4	4.2	3.0	5.6
18–24	11.6	9.5	4.9	2.6	2.7	5.1	13.9	12.0	3.1	4.6	3.2	12.0
24–36	21.2	18.5	3.3	8.2	10.9	20.3	16.0	22.7	5.5	17.7	13.3	22.7

* This plat received $NaNO_3$ at the rate of 240 pounds an acre in addition to the superphosphate for the first 4 years of the experiment.

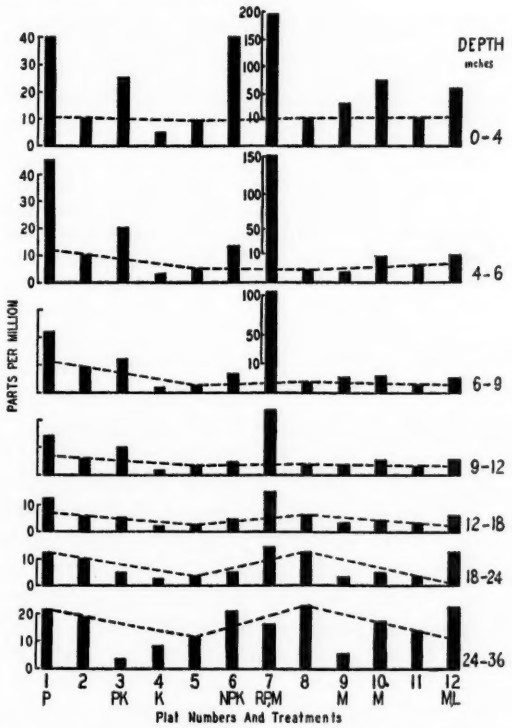


FIG. 1. EFFECT OF SOIL TREATMENTS ON THE EASILY SOLUBLE PHOSPHORUS OF THE SOIL AT VARIOUS DEPTHS

Broken line connects the control plats 2, 5, 8, 11

potash, in the plat treated with complete fertilizer, or in the plat treated with 5 tons of manure. The plat which received manure and lime accumulated some easily soluble phosphorus at all depths. Treatment with potash alone reduced the phosphorus below the corresponding control plat value at all depths. The plat treated with 2½ tons of manure exhibited accumulated phosphorus only at the surface (0–4 inches).

ACCUMULATION OF TOTAL PHOSPHORUS AND SOLUBILITY OF PHOSPHORUS FIXED IN THE SOIL

It was desired to ascertain the extent to which the total phosphorus of the soil of the various plats had been increased by the treatments and to determine the solubility of the fixed phosphorus. Accordingly total phosphorus was determined, and the results are presented in table 3.

These data were treated in the same manner as were those for easily soluble phosphorus, that is, control plat values were utilized in estimating the influ-

TABLE 3

Total phosphorus of the soil at various depths

Expressed as percentage of air-dry soil

DEPTH	PLAT* 1	PLAT 2	PLAT 3	PLAT 4	PLAT 5	PLAT 6	PLAT 7	PLAT 8	PLAT 9	PLAT 10	PLAT 11	PLAT 12
inches												
0–4	.050	.039	.044	.032	.034	.044	.089	.029	.031	.033	.031	.038
4–6	.055	.039	.042	.029	.029	.034	.068	.029	.034	.038	.032	.033
6–9	.039	.033	.032	.027	.023	.029	.042	.022	.022	.025	.020	.024
9–12	.038	.032	.032	.027	.024	.026	.027	.022	.023	.024	.022	.025
12–18	.031	.030	.024	.021	.019	.020	.018	.017	.018	.019	.018	.021
18–24	.027	.025	.019	.015	.014	.015	.015	.015	.013	.014	.013	.018
24–36	.024	.023	.013	.014	.016	.016	.019	.022	.020	.018	.016	.020

* For plat treatments, see table 2.

ences of treatments. For each plat treated with a phosphorus carrier the calculated increase of total phosphorus, if any, was expressed as percentage of the "control" value for the plat. The data are recorded in table 4.

These data reveal a much greater percentage accumulation of phosphorus in plat 7, treated with rock phosphate and manure, than in any other plat. The larger accumulation in this plat could probably be accounted for by the relatively large amount of phosphorus applied, if in no other manner. It is interesting to note, however, the accumulation is large only in the first 4 depths, or down to 12 inches. It is also of interest that plat 1, treated with superphosphate; plat 6, treated with a complete fertilizer; and plat 12, receiving manure and lime, showed greater percentage accumulations in the surface 4 inches than did plat 3, treated with superphosphate plus potash, or plats 9 and 10, receiving manure alone.

The superphosphate plat and the one treated with manure and lime exhibit

more consistent accumulations of phosphorus and to greater depths than do other fertilized plats of the group. Judging from the topography of the land and the total phosphorus values for control plat 2 as compared to control plats 5 and 8, it is probable that the superphosphate plat has more native phosphorus available to plants than do some of the other fertilized plats. This may account for some of the accumulation of phosphorus under this treatment, since the plants may have drawn less heavily on applied phosphorus on the more fertile soil. The application of $NaNO_3$ with the superphosphate, as noted in the discussion following table 1, during the first 4 years of the experiment may possibly have influenced downward movement and, hence, accumulation at lower depths. None of these lines of reasoning apply to the manure and lime plat, however. Natural soil variation may account for some of the apparent accumulation in this plat but hardly for all of it. It appears that liming not only brought about the retention of a comsiderable quantity of applied phosphorus in the soil but also aided its penetration.

TABLE 4

Accumulated (total) phosphorus as percentage of the native phosphorus of the soil

DEPTH	PLAT* 1	PLAT 3	PLAT 6	PLAT 7	PLAT 9	PLAT 10	PLAT 12
inches							
0–4	28	17	36	191	4	9	22
4–6	41	18	17	134	13	22	3
6–9	18	7	28	87	3	21	10
9–12	19	12	11	19	4	9	13
12–18	3	..	10	2	4	6	17
18–24	8	..	5	3	..	2	38
24–36	4	..	..	....	1	..	25

* For plat treatments, see table 2.

The low percentage accumulations in the surface of the plats treated with manure alone are striking. The same is true, to lesser extent, of the lower depths. It would appear that the phosphorus applied in the manure was less strongly absorbed by the soil and hence more completely utilized by the plants than that supplied in commercial fertilizers. This phosphorus may have remained in organic combination. Spencer and Stewart (6) have shown that certain organic phosphates retain a high degree of solubility and availability in the soil.

The percentages of phosphorus fixed in the soil of each plat in easily soluble form and in difficultly soluble form were determined by dividing the accumulated easily soluble phosphorus by the accumulated total phosphorus, each expressed as parts per million of air-dry soil. The difference between 100 and the percentage of easily soluble phosphorus represents that phosphorus which was fixed in difficultly soluble form. These data are recorded in table 5.

More of the accumulated phosphorus in plat 7, treated with manure and

rock phosphate, remained easily soluble than in any other plat. In the surface layer about 30 per cent remained easily soluble, and the corresponding values for the next 5 depths were, approximately, 40, 50, 45, 100, and 100 per cent. The more mobile portion of this phosphorus, therefore, retained the highest solubility, as all which moved into the 12–18-inch and the 18–24-inch layers remained easily soluble. The greater solubility of the residual phosphorus from this treatment may be due to either or both of two factors, namely, (*a*) the larger amount of accumulated phosphorus, perhaps exceeding the fixing capacity of the soil, and (*b*) the greater solubility of residual phosphorus from rock phosphate treatment than from superphosphate treatment in a dilute "strong-acid" extracting reagent, such as the Truog reagent. Morgan (3) has presented data indicating greater solubility of residual phosphorus from rock phosphate than from superphosphate, in such an extracting reagent.

TABLE 5

Percentages of phosphorus fixed in easily soluble and difficultly soluble form

DEPTH	PLAT* 1		PLAT 3		PLAT 6		PLAT 7		PLAT 9		PLAT 10		PLAT 12	
	A†	B‡	A	B	A	B	A	B	A	B	A	B	A	B
inches														
0–4	29	71	24	76	27	73	32	68	40	60	51	49	16	84
4–6	22	78	19	81	17	83	38	62	..	..	4	96	40	60
6–9	22	78	23	77	6	94	52	48	32	68	6	94	6	94
9–12	14	86	14	86	5	95	46	54	5	95	11	89	9	91
12–18	65	35	..	..	5	95	100	0	..	..	2	98	9	91
18–24	11	89	..	..	..	..	100	0	33	67	..	..	18	82
24–36	27	73	..	..	..	..	....	..	..	..	..	..	24	76

* For plat treatments, see table 2.

† A = easily soluble.

‡ B = difficultly soluble.

The superphosphate plat 1 ranked second to the manure and rock phosphate plat, when the entire profile is considered, in maintaining the fixed phosphorus in an easily soluble form. The superphosphate and potash plat 3 was a close third. The two manure plats 9 and 10 had a considerable proportion of their accumulated phosphorus in an easily soluble form in the surface layer, but at lower depths the residual phosphorus was largely difficultly soluble. This was especially true of the heavier manure treatment. The complete fertilizer plat 6 and plat 12, treated with manure and lime, showed a surprisingly large proportion of the accumulated phosphorus in a difficultly soluble form. Weiser (8), however, has indicated that lime may increase the fixing power of iron and aluminum compounds, at least under certain conditions.

A relatively large proportion of the residual phosphorus of all treatments, excepting rock phosphate and manure, was fixed in difficultly soluble form. The removal of the easily soluble phosphorus by the growing alfalfa plants, however, may account for this situation.

EFFECT OF FERTILIZER RESIDUES IN THE SOIL ON BASE EXCHANGE CAPACITY AND ULTIMATE pH VALUES

Mattson and Hester (1) have shown in laboratory experiments that addition of phosphorus to soils, particularly to the clay fraction, increases the base exchange capacity of the soil. This supposedly results from an increase in the acidoid portion of the soil through the absorption of $PO_4^{\equiv}$. As a further consequence of this build-up of the acidoid portion the so-called ultimate pH (pH value of the H^+-saturated soil) is lowered.

Base exchange capacity was determined for the samples from the first five depths of each plat and also for the two lowest depths of plats 8, 11, and 12. The data are presented in table 6. By utilizing the values for control plats 2, 5, 8, 11 in a manner similar to that illustrated in figure 1 for easily soluble phosphorus data, the effects which treatments have produced upon the base exchange capacity of the soil at the various depths were determined.

TABLE 6

Base exchange capacity of the soil at various depths

Expressed as milligram equivalents per 100 gm. of air-dry soil

PLAT* NUMBER	0-4 INCHES	4-6 INCHES	6-9 INCHES	9-12 INCHES	12-18 INCHES	18-24 INCHES	24-36 INCHES
1	22.18	20.40	22.86	23.57	26.54	.....	.....
2	19.43	20.21	21.40	22.54	24.93	.....	.....
3	18.93	18.14	20.26	21.07	22.71	.....	.....
4	17.90	18.40	18.54	20.43	21.28	.....	.....
5	17.11	18.26	19.40	19.90	20.43	.....	.....
6	15.97	16.90	18.57	19.24	20.10	.....	.....
7	16.53	16.21	18.18	18.96	19.50	.....	.....
8	15.68	16.50	18.76	19.50	19.33	18.43	17.36
9	16.43	17.54	18.07	18.28	18.67	.....	.....
10	17.00	17.07	18.21	18.81	19.07	.....	.....
11	15.21	16.14	17.64	18.10	19.07	17.57	15.71
12	19.21	18.33	19.07	20.43	21.33	21.30	19.61

* For plat treatments, see table 2.

In the surface 4 inches only the superphosphate, among the commercial fertilizer treatments, is indicated to have increased the base exchange capacity of the soil. Since plat 1 contains 45,400 pounds of organic carbon per acre in the surface $6\frac{2}{3}$ inches of soil in comparison with 37,800 pounds in control plat 2, it is possible this factor may account for at least a considerable part of the difference in the exchange capacities. The manure treatments, plats 9, 10, 12, increased the exchange capacity of the soil, apparently to the greatest extent in the case of the lime and manure treatment, plat 12.

In the 4–6-inch layer all commercial fertilizers produced apparent decreases of exchange capacity, whereas the manure treatments continued to indicate increases. At lower depths, only the superphosphate plat and the lime and manure plat showed what appear to be appreciable increases. In the super-

phosphate plat the higher organic matter content of the surface soil, already noted, may also prevail at lower depths and may account, at least in part, for a high exchange capacity. The manure and lime plat, however, contains 38,800 pounds of organic carbon in its surface soil, which is less than the amount in plat 7, treated with manure and rock phosphate, or in plat 10, receiving 5 tons of manure, and is only 2,300 pounds more than the carbon of plat 11, the adjoining control plat. It would appear, therefore, that the manure and lime treatment increased the exchange capacity of the soil and that the influence extended throughout the profile. It might be supposed that this increase could result from greater root residues from the alfalfa plants in the plat treated with lime. Such would hardly seem to be the case, however, since both the plat treated with 5 tons of manure and the manure and rock phosphate plat produced more alfalfa hay than did the manure and lime plat.

TABLE 7

Effect of electrodialysis on base exchange capacity of soil from the surface layer (0–4 inches)

PLAT* NUMBER	ULTIMATE pH	EXCHANGE CAPACITY, NORMAL	EXCHANGE CAPACITY AFTER ELECTRODIALYSIS	REDUCTION OF EXCHANGE CAPACITY
		m.e.†	*m.e.*†	*m.e.*†
1	4.0	22.18	21.0	1.18
2	4.2	19.43	19.3	0.13
3	4.1	18.93	18.07	0.86
4	4.2	17.90	17.43	0.47
5	4.2	17.11	16.71	0.40
6	4.15	15.97	15.57	0.40
7	4.1	16.53	16.40	0.13
8	3.9	15.68	15.51	0.17
9	3.8	16.43	16.35	0.08
10	4.0	17.00	15.93	1.07
11	4.1	15.21	14.79	0.42
12	4.1	19.21	15.71	3.50

* For plat treatments, see table 2.

† Per 100 gm. of air-dry soil.

By the methods of camparison employed in the preceding paragraphs, fertilizer residues are indicated to have produced comparatively small effects upon the exchange capacity of the soil, and some of the treatments resulted in reductions. It seemed desirable, therefore, to try another method of approach, particularly for the surface samples. Accordingly, the 0–4-inch samples were electrodialyzed with a three-compartment Bradfield cell until, upon continuing electrodialysis 3 hours with a fresh charge of water in each compartment, the dialyzate in the cathode chamber produced but very faint color with phenolphthalein. Then the ultimate pH value and the base exchange capacity of the electrodialyzed soil were determined. The data are recorded in table 7.

The ultimate pH values range between the narrow limits 3.8 and 4.2, and the variation appears to bear no relation to treatment. It may be concluded,

therefore, that such residues as were left in these plats from the soil treatments did not appreciably affect the ultimate pH values. Turning then to the base exchange data, one will note that the greatest decreases of exchange capacity as a result of electrodialysis occurred in the plats treated as follows: superphosphate (plat 1), 5 tons of manure annually (plat 10), lime plus $2\frac{1}{2}$ tons of manure annually (plat 12). Of these plats, the decrease for plat 12 was three or more times that of either plat 1 or plat 10. These three plats are among the four or five plats in the series having the highest organic carbon content. The lime and manure plat, however, has less organic carbon than the plat treated with 5 tons of manure. Hence it is indicated that new exchange complexes were created by the action of the lime, or the lime and manure together, which were decomposed in the electrodialysis process. It is possible that phosphorus residues may have acted in somewhat similar fashion, since the reductions in exchange capacity for the superphosphate, and the superphosphate plus potash plats, though small, were larger than the values for plats receiving no phosphorus. The complete fertilizer plat did not behave thus, however, and there is therefore no convincing evidence that new exchange complexes, in appreciable quantity, were built up by the phosphorus residues. In the manure and rock phosphate plat 7, where treatment increased the total phosphorus of the soil by 191 per cent, only a very small reduction of exchange capacity upon electrodialysis is indicated. This could probably be accounted for by the fact that the soil did not strongly absorb the phosphorus from rock phosphate. Prince and Toth (4) have presented data indicating appreciable increases in exchange capacities of certain soils in cylinder experiments as a result of superphosphate applications. These New Jersey soils had low exchange capacities, and the superphosphate applications were much heavier than those in the work reported here. In other work reported by the same authors (5) only small reduction in exchange capacity occurred upon electrodialysis of soils to which mineral fertilizers were applied alone, but where these same fertilizers were supplemented with lime much greater reductions were indicated.

SODIUM RESIDUES FROM SODIUM NITRATE AND THE EFFECT OF SODIUM AND LIME ON pH VALUES

Sodium nitrate was applied annually to plat 6 of this experiment at the rate of 240 pounds an acre. Lime was applied to plat 12 at the rate of 1000 pounds of hydrated lime per acre as needed to maintain a pH value of at least 6.5. Although pH values were determined on all samples and sodium was determined in the first four depths for all plats, the effects of treatments with the sodium salt are sufficiently illustrated in the data for certain plats as indicated in table 8.

Sodium accumulated to only a small extent in plat 6, treated with $NaNO_3$. There was appreciable accumulation, however, and the data indicate that the sodium moved downward, probably readily. Some of it was undoubtedly

leached below a depth of 3 feet. The greatest accumulation occurred in the 12–18-inch and 18–24-inch levels. The accumulated sodium appears to have influenced the pH values, particularly below the 6-inch level. The data do not indicate an accumulation of active sodium sufficient to influence the physical condition of the soil. Penetration and plowing tests, measured qualitatively, do not indicate any detrimental influence.

Liming (plat 12) affected the pH values, it appears, to a depth of 18 inches. The data for the samples taken in 1923 when the experiment had been under way only 14 years (2) indicated no influence of liming on the pH values below 6 inches. The data for phosphorus reported herein point toward an influence of the lime applications throughout the 3 feet of soil sampled.

TABLE 8

Exchangeable sodium and the effects of sodium and lime on pH values*

DEPTH	PLAT† 2		PLAT 3		PLAT 6		PLAT 11	PLAT 12
	Na	pH	Na	pH	Na	pH	pH	pH
inches								
0–4	.15	5.84	.18	5.75	.42	5.69	5.88	6.52
4–6	.09	5.75	.10	5.58	.46	5.58	5.54	6.14
6–9	.17	5.67	.19	5.54	.66	5.96	5.50	6.05
9–12	.10	5.58	.12	5.62	.84	5.97	5.50	6.01
12–18	...	5.58	.15	5.88	.95	6.12	5.69	6.01
18–24	...	5.58	.17	5.92	.95	6.22	5.92	5.92
24–36	...	5.84	.22	5.98	.88	6.24	6.05	6.09

* Milligram equivalents per 100 gm. of air-dry soil.

† For plat treatments, see table 2.

POTASSIUM RESIDUES AND THE FIXATION OF POTASSIUM IN EXCHANGEABLE AND NONEXCHANGEABLE FORM

Potassium as K_2SO_4 was applied to three plats in the experiment. On plat 4 potash was applied alone, on plat 3 it was applied with superphosphate, and on plat 6 with superphosphate and $NaNO_3$. The manured plats received liberal amounts of potash, since the manure used contained, as an average, 18.7 pounds of potassium per ton of fresh manure.

The exchangeable potassium for the first five depths is shown in table 9. From these data it may be observed that potassium did not accumulate below 12 inches. Plat 10, treated with 5 tons of manure, accumulated the greatest quantity of exchangeable potassium in the surface soil, and plat 4, receiving potash alone, and plat 12, treated with manure and lime, stood second in this respect. In the 4–6-inch layer the heavier manure treatment and the potash treatment again stand out. The potash plat and plat 3, treated with potash and superphosphate, were the only ones which showed appreciable accumulations in the 6–9-inch layer. In the 9–12-inch layer the only appreciable accumulation occurred in the potash plat.

A limited number of determinations of total potassium were made. The results, though not conclusive, indicate that treatments with potash carriers maintained, or increased, the exchangeable potassium more effectively than the total potassium. Though alfalfa did not respond to potash treatments on this soil, it is well known that plants "luxuriously" absorb potassium from a soil in which the supply is abundant. The limited data also indicate that in those plats in which accumulations of total potassium occurred 77 to 96 per cent of the accumulated total potassium was fixed in nonexchangeable form.

TABLE 9

Exchangeable potassium at various depths in all plats and the accumulated exchangeable potassium of plats treated with potash carriers

PLAT* NUMBER	0-4 INCHES		4-6 INCHES		6-9 INCHES		9-12 INCHES		12-18 INCHES	
	A†	B‡	A	B	A	B	A	B	A	B
1	0.89		0.85		0.87		.58		.76	
2	1.14		1.05		0.94		.87		.87	
3	1.32	0.18	1.05	.10	1.09	.20	.65		.71	
4	1.73	0.60	1.29	.43	1.13	.28	.91	.18	.72	
5	1.12		0.77		0.80		.66		.66	
6	1.35	0.29	0.65		0.69		.63		.69	.02
7	1.06	0.06	0.72	.01	0.66		.74	.07	.59	
8	0.94		0.68		0.72		.67		.68	
9	1.41	0.48	0.83	.13	0.59		.49		.69	
10	1.98	1.07	1.11	.38	0.59		.56		.59	
11	0.90		0.75		0.59		.70		.78	
12	1.51	0.61	0.90	.15	0.64	.05	.68		.79	.01

* For plat treatments, see table 2.

† A—Exchangeable potassium, in milligram equivalents per 100 gm. of air-dry soil, as determined by analysis.

‡ B—Accumulated exchangeable potassium, in milligram equivalents per 100 gm. of air-dry soil, resulting from treatments.

SUMMARY

A study has been made of the nature, extent, and distribution of fertilizer residues in the soil of an old fertility experiment at the Kansas Agricultural Experiment Station. The experiment involved the continuous growing of alfalfa, and the fertilizers were applied as top-dressings for 27 years. Emphasis was placed on the study of phosphorus residues; sodium and potassium were also studied.

A large proportion of the easily soluble phosphorus which accumulated in the soil as a result of treatment with various phosphorus carriers was found in the surface 4 inches of soil. There appeared to be accumulated phosphorus to depths of 2 to 3 feet, however, in plats treated with rock phosphate and manure and with lime and manure and, to lesser extent in the superphosphate plat. Potash or potash and sodium nitrate applied with superphosphate

resulted in more complete utilization of the phosphorus by the plants than did superphosphate used alone.

When total phosphorus was studied, it was found that in the surface 4 inches of soil and in immediately succeeding depths accumulations amounted to from 3 to 191 per cent of the native phosphorus of the soil. Lime appeared to have brought about the retention of more of the phosphorus applied in the form of manure than where manure was applied alone, and at the same time it aided phosphorus penetration. In general, phosphorus applied in manure was largely utilized by the plants, and little accumulated.

Where superphosphate was applied, alone or supplemented, 70 per cent or more of the accumulated phosphorus was fixed in difficultly soluble form. Rock phosphate was much less strongly fixed, and at depths of 1 to 2 feet the accumulated phosphorus was entirely soluble in dilute acid. Except in the surface 4 inches of soil, phosphorus residual from manure was very largely fixed in difficultly soluble form.

The data afforded no convincing evidence that residual phosphorus increased the base exchange capacity of the soil. Lime and manure treatment brought about an appreciable increase, however, and this increased exchange capacity disappeared when the soil was electrodialyzed. Ultimate pH values were unaffected by the phosphorus treatments.

Exchangeable sodium content of the soil was increased somewhat by a treatment involving $NaNO_3$, but most of the accumulation was at depths of $1\frac{1}{2}$ to 3 feet.

The potash treatments, which were light, left only very small residues, and these were confined to the upper 9 inches of soil. Exchangeable potassium appeared to have been maintained, or accumulated, more effectively than did total potassium. Limited data indicated that where total potassium was increased as a result of treatments, 77 to 96 per cent of the accumulated potassium was fixed in nonexchangeable form.

REFERENCES

(1) MATTSON, S., AND HESTER, J. B. 1935 The laws of soil colloidal behavior: XV. The degradation and the regeneration of the soil complex. *Soil Sci.* 39: 75–84.

(2) METZGER, W. H. 1934 Distribution of fertilizer residues in the soil after fourteen years of a fertilizer experiment with alfalfa. *Jour. Amer. Soc. Agron.* 26: 620–625.

(3) MORGAN, M. F. 1935 The variance of methods of testing for phosphorus on soils previously treated with different phosphate fertilizers. *Proc. First Ann. Meeting Comm. on Fert. Amer. Soc. Agron.* pp. 45–51.

(4) PRINCE, A. L., AND TOTH, S. J. 1936 The effect of phosphates on the cation exchange capacity of certain soils. *Soil Sci.* 42: 281–290.

(5) PRINCE, A. L., AND TOTH, S. J. 1937 Electrodialysis and cation exchange studies on soils of varying organic matter content. *Soil Sci.* 43: 205–217.

(6) SPENCER, V. E., AND STEWART, R. 1934 Phosphate studies: I. Soil penetration of some organic and inorganic phosphates. *Soil Sci.* 38: 65–79.

(7) TRUOG, E. 1930 The determination of the readily available phosphorus in soils. *Jour. Amer. Soc. Agron.* 22: 874–882.

(8) WEISER, V. L. 1933 Fixation and penetration of phosphates in Vermont soils. Vt. Agr. Exp. Sta. Bul. 356.

CHEMICAL NATURE OF ORGANIC MATTER IN DIFFERENTLY CROPPED ARID SOILS[1]

KENNETH R. STEVENS[2]

Utah Agricultural Experiment Station

Received for publication July 11, 1938

A proximate system of organic matter analysis developed and used for studying the processes of decomposition of plant materials (4), and applied to the analysis of peat and of forest and inorganic soils (2, 3, 5), has been applied in the present study to the analysis of a western arid soil. This system of analysis accounts for the major part of the organic constituents of such organic residues and soils. The groups thus accounted for consist of the ether- and alcohol-soluble portion; the carbohydrate fraction, measured as reducing sugar; the organic nitrogenous complexes; and the lignin-humus.

Such a method may not be recommended for general routine purposes, but it is an important aid to the soil investigator in studying the chemical nature and makeup of the organic matter of the soil. A further advantage comes from the opportunity to classify by grouping the various constituents of the soil organic matter into their respective fractions.

Results obtained in the analysis of inorganic soils (3) indicate that the chemical nature of the organic matter is different in various soils and even in two differently treated experimental plats which were located on the same type of soil at the New Jersey Experiment Station farms at New Brunswick.

SOILS

An attempt was made in this study to determine the extent to which this method would show differences in the nature of the organic matter in some experimental plats at the Greenville Experimental Farm, north of Logan, Utah, which is under irrigation. This farm is situated on a sandy loam soil, which is unusually uniform to a depth of more than 10 feet. The soil is well aerated, has good drainage (with a corresponding low water table), is high in calcium and magnesium (mainly as carbonates), and is well supplied with phosphorus and potassium. Nitrogen occurs in small quantities. The soil is very productive. The ammonifying, the nitrifying, and the nitrogen-fixing powers of the soil are above the average, and an interesting bacterial flora exists.

[1] Contribution from the department of chemistry and bacteriology, Utah Agricultural Experiment Station.

[2] Associate bacteriologist.

SAMPLING

Soil samples were taken from plats which had supported continuous fallow, oats, and alfalfa, respectively, for 25 years. A considerable amount of grass was growing on the alfalfa plat. This is typical, however, of the farms in this region where soils are cropped indefinitely to alfalfa. The surface soil was removed to a depth of one-half inch, and samples were taken by auger from the first-, second-, and third-foot levels. Many borings were made and composited to make up the sample representing each respective plat and depth. All samples were mixed well, dried, and sieved.

METHODS

Air-dried soil was used for the analyses, but all results were calculated on the oven-dried basis. Total nitrogen determinations were made according to the official Gunning-Kjeldahl method on 5-gm. portions of soil. Total carbon and inorganic carbon determinations were made according to the official methods of the Association of Official Agricultural Chemists. Reducing sugar was determined by a combination of the Munson and Walker and Bertrand methods as outlined by Mathews (1). The total organic matter of the soil samples, obtained by multiplying the organic carbon content of the soil by the factor 1.724, was the basis for calculating the results of the organic matter analysis.

The procedure was as follows: Two 200-gm. quantities of the air-dried soil were placed in 500-cc. Erlenmeyer flasks and extracted with hot 95 per cent alcohol for 2 hours on a water bath. The extract was evaporated in weighed dishes and the soluble fraction determined gravimetrically. The alcohol-treated soil was next extracted with anhydrous ether for 12 to 16 hours. The ether-soluble fraction was also evaporated in weighing bottles and dried to constant weight.

Two 40-gm. portions of the dried residual soil were placed in beakers and treated with enough 2 per cent hydrochloric acid to neutralize the carbonates completely. This acid and subsequent washings were removed by decantation. The soil was allowed to dry in the beakers. The beakers with soil were placed in an ice bath, and the soil was treated with 25 cc. of hydrochloric acid solution (sp. gr. 1.115) for $2\frac{1}{2}$ hours. Next, 250 cc. of distilled water was added to each beaker, and the diluted acid solution was heated in the autoclave at 15 pounds pressure for 1 hour. The supernatant solution was passed through weighed filter paper, and the soil was washed free from acid by decantation. The acid filtrate and the first two washings were retained, made up to volume, and the amount of reducing sugar was determined. The sugar content became an index of the amount of carbohydrate material present in the soil organic matter soluble in the acid hydrolysis.

After all samples were freed from acid, the soil residue was washed into the weighed filters and dried. Two 5-gm. samples from each residue were used

for total nitrogen determinations, and two 2-gm. samples were used for total carbon determinations. The "lignin-humus" complex was calculated from the carbon and nitrogen content of this residue. The following formula was used for this purpose (3, p. 105):

$$\text{Per cent of "lignin-humus" complex in soil} = \frac{a \times 100}{A} - \frac{b \times 100}{S}$$

where a = the carbon content in the acid residue, calculated on the basis of the total original sample of soil,

A = the total carbon content of the sample of soil,

b = protein content in the acid residue, obtained by multiplying the nitrogen content of the residue by 6.25; this is then calculated for the whole sample,

S = total organic matter in the soil sample, as calculated from the organic carbon of the soil.

RESULTS

The results of the proximate chemical composition of the organic matter in these soils, calculated on the basis of the total soil organic matter as determined from the total organic carbon figures given in table 1, are given in table 2.

The results support work done elsewhere, which shows that this method can be used on inorganic soils and will account for the major portion of the soil organic matter in rather definite groups (3). The soils studied are somewhat above the average of some soils from less arid regions (3) in the ether- and alcohol-soluble fraction. They are extremely low in the carbohydrate fraction, giving only slightly better than traces of this group. The content of "protein" is high, and the carbon-nitrogen ratio is narrow, a condition which indicates an advanced degree of decomposition of the organic residues added to the soil. The "lignin-humus" is also high.

Of the three plats, the fallow plat has the lowest average for the various fractions, except the "protein." This explains the fact that it has the lowest average carbon-nitrogen ratio of the three plats. Every fraction, except the "lignin-humus" complex, shows a decrease with depth. No plant growth occurred on this plat during 25 years; however, the first 3 feet of this soil are known to be high in power to fix nitrogen, to decompose proteins, and to accumulate nitrates. The rapid and extensive activity of the rich microflora would presumably account for the very narrow carbon-nitrogen ratio found.

Of the three plats, the oats plat has the highest percentage for the ether- and alcohol-soluble, the "protein," and the "lignin-humus" fractions in the first foot horizon. It averages highest of the three plats for the 3 foot levels in the carbon-nitrogen ratio. The annual plowing under of the stubble and fibrous roots, both high in the carbon-nitrogen ratio, may largely account for this.

The alfalfa plat shows the smallest decrease in the fractions with depth. This indicates the influence of the particular type of root system common

to this crop. Whereas the fallow and oats plats showed a decrease in the "protein" fraction with depth, this plat increased in protein. The "lignin-humus" fraction decreased with depth in the alfalfa and oats plats, whereas it increased in the fallow plat.

TABLE 1

Carbon and nitrogen relationships in the soil organic matter on the basis of dry soil

CROP AND HORIZON	ORIGINAL SOIL				ACID-HYDROLYZED SOIL		
	Organic matter C×1.724	Total organic carbon	Total nitrogen	C/N	Total carbon*	Total nitrogen	C/N
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	
Fallow:							
First foot	1.41	0.82	0.110	7.45	0.43	0.145	2.97
Second foot	1.34	0.78	0.092	8.48	0.41	0.122	3.36
Third foot	1.09	0.63	0.068	9.26	0.37	0.106	3.49
Oats:							
First foot	2.33	1.35	0.154	8.77	0.81	0.210	3.86
Second foot	1.50	0.87	0.095	9.16	0.48	0.059	8.14
Third foot	1.03	0.60	0.065	9.23	0.32	0.030	10.67
Alfalfa:							
First foot	2.31	1.34	0.150	8.93	0.80	0.206	3.88
Second foot	1.78	1.03	0.116	8.88	0.61	0.158	3.86
Third foot	1.21	0.70	0.085	8.24	0.41	0.124	3.31

* Calculated on the basis of original sample.

TABLE 2

*Proximate composition of the soil organic matter on the basis of organic matter content of soil**

CROP AND HORIZON	ETHER- AND ALCOHOL-SOLUBLE FRACTION	CARBOHYDRATE FRACTION, AS SUGAR	"PROTEIN"	"LIGNIN-HUMUS"	SUM OF ORGANIC MATTER ACCOUNTED FOR
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Fallow:					
First foot	2.80	0.10	48.79	46.01	97.70
Second foot	1.95	0.07	42.91	46.87	91.80
Third foot	1.74	0.06	39.00	52.65	93.45
Oats:					
First foot	3.35	0.14	41.33	54.36	99.18
Second foot	2.14	0.08	39.60	52.71	94.53
Third foot	1.63	0.06	39.42	51.50	92.61
Alfalfa:					
First foot	3.15	0.22	40.61	54.12	98.10
Second foot	2.53	0.12	41.01	53.67	97.33
Third foot	2.14	0.09	43.88	52.17	98.28

* The organic matter content is calculated by multiplying the organic carbon by the factor 1.724.

SUMMARY

The method of proximate analysis of soil organic matter was applied to an arid soil under irrigation.

Samples were taken from adjacent plats which had supported continuous fallow, oats, and alfalfa, respectively, for 25 years.

The results showed these soils to be somewhat high in the ether- and alcohol-soluble fraction. The "protein" and "lignin-humus" fractions were high. The carbohydrate fraction was unusually low.

The ether- and alcohol-soluble and carbohydrate fractions decreased with depth for the three plats. The "protein" fraction decreased with depth for the fallow and oats plats but increased with depth for the alfalfa plat. The "lignin-humus" fraction increased with depth in the fallow plat but decreased with depth in the oats and alfalfa plats.

REFERENCES

(1) MATHEWS, A. P. 1925 Physiological Chemistry. Wm. Wood and Co., New York.

(2) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: I. *Soil Sci.* 26: 113–137.

(3) WAKSMAN, S. A., AND STEVENS, K. R. 1930 A critical study of the methods for determining the nature and abundance of soil organic matter. *Soil Sci.* 30: 97–116.

(4) WAKSMAN, S. A., AND TENNEY, F. G. 1927 Composition of natural organic materials and their decomposition in the soil: I and II. *Soil Sci.* 24: 275–283, 317–333.

(5) WAKSMAN, S. A., TENNEY, F. G., AND STEVENS, K. R. 1928 The rôle of microörganisms in the transformation of organic matter in forest soils. *Ecology* 9: 126–144.

A MICROCHEMICAL STUDY OF THE EFFECTS OF BORON DEFICIENCY IN COTTON SEEDLINGS[1]

CECIL H. WADLEIGH AND JOHN W. SHIVE

New Jersey Agricultural Experiment Station

Received for publication July 6, 1938

The marked morphological effect of boron deficiency upon the growing points of plants has been noted many times (1, 4, 9, 10), but information concerning the physiological disturbances which accompany the morphological transformation is still meager. The following study was undertaken for the purpose of following, microchemically, a few of the physiological and chemical changes which take place in the cells and tissues of the growing point as boron deficiency symptoms develop.

Seed of the Qualla variety of cotton was germinated in white sand. Upon germination, these seedlings were supplied with a nutrient solution of the following basic composition:

Salt	*Molar Concentrations*
KH_2PO_4	.0025
$CaCl_2$	.0025
$MgSO_4$	.0020
$NaNO_3$	.0040

Manganese and iron were also supplied as manganese sulfate and ferric tartrate, respectively, at the rate of 0.5 p.p.m. of solution. The solution applied to part of these cultures contained no boron other than that which might have been present in the distilled water or in the Baker's analyzed salts that were employed. Plants in the remaining cultures of this study received the solution described, except that boron was added as H_3BO_3 at the rate of 1 p.p.m. In order to follow changes which occurred at the inception of boron deficiency symptoms and as these symptoms progressed, sections for microchemical study were taken, from time to time, from the stem tips of plants grown in both types of solution.

The methods followed in this study are all found in the microchemical outlines of Eckerson[2] and Sampson[3] with the exception of the procedure for estimating the pH of the cells. These determinations were made in accordance with the methods of Small (8) and Rogers and Shive (7).

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

[2] Eckerson, S. H. Plant Microchemistry. (Mimeographed outline.)

[3] Sampson, H. C. Plant Microchemistry. (Mimeographed outline.)

EXPERIMENTAL OBSERVATIONS

The approximate pH values of the tissues of the stem tip of a cotton seedling grown in a complete culture solution, as summarized from observations on numerous plants, were as follows.

Tissue	*pH Range*
Phloem	6.0–6.2
Xylem	4.2–4.6
Endodermis	4.0–4.4
Cortex	5.8–6.0
Pith	6.2–6.4
Bast Fibers	3.8–4.0

With the development of boron deficiency symptoms, the acidity of certain tissues in the stem tip appeared to increase, but not uniformly in all cells of a given tissue. The first change observed in the boron deficient plants was the occurrence of a few scattered cells in the pith and cortex which were pronouncedly more acid (pH 3.8–4.4) than the other cells in these tissues (pH 5.8–6.4). This observation was made before any necrosis was apparent either externally or internally. As boron deficiency became more severe the proportion of these very acid cells increased, and such cells began to appear in the pericycle and in the older regions of the xylem parenchyma. When the major portions of the pith cells were showing this abnormally acid reaction, a breaking down of the pith cell walls, accompanied by a deep brown color, began to appear. When decomposition of the pith cells was well under way certain cells scattered throughout the phloem and young xylem parenchyma showed relatively high acidity. This acidification of the cells continued, and eventually there was evidence of a breaking down of cell organization in the phloem and in the regions of the young xylem.

In connection with these acidity changes, there was a marked increase in carbohydrate content of the boron deficient plants. Fructose could not be detected in the control plants; but in the boron deficient plants, slight fructose accumulation was indicated, and as boron deficiency became more severe, the test for fructose became more pronounced. With the development of boron deficiency symptoms, both glucose and sucrose, likewise, increased markedly. Even when the pith cells had begun to break down there was, as yet, however, no marked accumulation of starch. At this stage, starch was apparent only in the endodermis of the plants, but it was present in these cells of the diseased plants in much greater abundance than in the corresponding cells of the control plants. The accumulation usually associated with boron deficiency, as pointed out by Johnston and Dore (3), probably follows severe boron starvation and is due to the condensation of sugars which *first* accumulated as a result of the inability of the meristematic tissues of the plant to utilize these products in the metabolic processes.

In connection with the study of these sugar accumulations, tests for nitrogenous substances in the cells of the stem tips brought out some interesting

relationships. The nitrate nitrogen content of the boron deficient plants was much lower than that of the control plants. As observations on the roots of boron starved plants showed that the growing points were either dying or dead, undoubtedly the lower nitrate nitrogen content of the boron deficient plants was due to the inability of the roots to absorb the nitrate ion rather than to its increased utilization within the plant.

One of the most significant observations made on these plants was the very marked accumulation of ammonium nitrogen in the boron deficient plants. The plants received no ammonium nitrogen in the nutrient solution, and only a relatively small amount was found in scattered cells of the more active meristematic regions of the stem tip. As the boron deficiency symptoms developed, the test for ammonium content of the plants with severe boron deficiency symptoms was extremely high. In fact, this observation was so striking that the ammonium content was checked by three different technics and with several different plants. The ammonium nitrogen did not accumulate uniformly in all cells but appeared in intense concentrations in scattered cells throughout the tissue. These high ammonium cells were found to appear first in the pith and cortex. As boron deficiency symptoms became more severe, the high ammonium cells increased in number and became apparent in the xylem parenchyma and phloem tissues. It appeared to be very significant that these ammonium accumulations occurred in the same cells which were previously referred to as becoming more acid in reaction than the rest of the cells of the same tissue.

That both sugars and ammonium nitrogen accumulate in the same tissues of the same plant is worthy of note and strongly suggests that boron deficiency inhibits or at least retards normal protein synthesis, since Murneek (6) finds that: "When ammonium salts are fed to leaves high in carbohydrates, proteins will be formed quickly; when carbohydrates are short, asparagine will be produced; and when carbohydrates are absent, ammonia will accumulate in the cells until poisoning of the leaves occurs."

Necrosis did develop in the stem tips, but no definite statement can be made as to the rôle the ammonium accumulations play in the lethal activities of the dying cells. The evidence strongly suggests that the metabolic disturbances arising from boron deficiency include a decreased rate of oxidation of sugars, a decreased rate of aminization of carbohydrate derivatives, and an inbibition of the production of proteinaceous compounds essential for the preservation of healthy protoplasm. These processes are fundamental in protein metabolism and, hence, in metabolic activity in meristematic regions.

In this latter connection, tests for proteinaceous substances brought out some interesting points. In the control plants, it was impossible to get a test for proteins with Millon's reagent, the xanthoproteic test, or the biuret reaction unless the tissue sections were first treated with ether and alcohol to denature the proteins. This is the characteristic response of healthy protoplasm. As boron deficiency symptoms became more marked, however,

these protein tests were obtained without the aid of a denaturant. Such a test is characteristic of degenerating protoplasm. Pronounced tests for degenerated proteins were observed in the scattered cells which showed the unusually high acid reaction and high ammonium tests.

The actual function of boron in metabolic processes still remains open to conjecture. It is known (5), however, that boron has a pronounced affinity for oxygen and nitrogen, and may become very active in certain oxidation-reduction systems. The observed anomolous biochemical conditions in the boron deficient plants suggest extreme disturbances in the normal oxidation-reduction equilibria within the cells. Boron is also known to have considerable affinity for the hydroxy groups of polyhydric alcohols. This latter relation has been pointed out (2, 3) in connection with the disturbed carbohydrate metabolism accompanying boron deficiency.

SUMMARY

The development of boron deficiency symptoms in cotton seedlings was followed microchemically. As the symptoms increased in severity, scattered cells throughout the tissues of the stem tips were observed to become much more acid than the normal cells of these tissues. Ammonium nitrogen was observed to accumulate, especially in the more acid cells, although no ammonium nitrogen was supplied to these plants. Sugars were found to accumulate. Protein tests indicated a progressive degeneracy of the protoplasm. It is suggested that in the absence of boron the normal course of protein synthesis is altered.

REFERENCES

(1) BRENCHLEY, W. E., AND WARINGTON, K. 1927 The rôle of boron in the growth of plants. *Ann. Bot.* 41: 167–189.

(2) EATON, F. M. 1935 Boron in soils and irrigation waters and its effect on plants. *U. S. Dept. Agr. Tech. Bul.* 448.

(3) JOHNSTON, E. S., AND DORE, W. H. 1929 The influence of boron on the chemical composition and growth of the tomato plant. *Plant Physiol.* 4: 31–62.

(4) MCHARGUE, J. S., AND CALFEE, R. K. 1933 Further evidence that boron is essential for the growth of lettuce. *Plant Physiol.* 8: 305–315.

(5) MELLOR, J. W. 1924 A Comprehensive Treatise on Inorganic and Theoretical Chemistry, vol. 5. Longmans, Green and Co., London.

(6) MURNEEK, A. E. 1935 The physiological rôle of asparagine and related substances in nitrogen metabolism of plants. *Plant Physiol.* 10: 447–465.

(7) ROGERS, C. H., AND SHIVE, J. W. 1932 Factors affecting the distribution of iron in plants. *Plant Physiol.* 7: 227–252.

(8) SMALL, J. 1929 Hydrogen Ion Concentration in Plant Cells and Tissues. Borntraeger Bros., Berlin.

(9) SOMMER, A. L., AND SOROKIN, H. 1928 Effect of the absence of boron and some other essential elements on the cell and tissue structure of the roots of *Pisum sativum*. *Plant Physiol.* 3: 237–261.

(10) WARINGTON, K. 1926 The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. *Ann. Bot.* 40: 27–43.

THERMOPHILIC ACTINOMYCETES AND FUNGI IN SOILS AND IN COMPOSTS[1]

SELMAN A. WAKSMAN, W. W. UMBREIT, AND T. C. CORDON

New Jersey Agricultural Experiment Station

Received for publication July 29, 1938

The optimum temperature for the growth of the great majority of the forms of life which inhabit natural substrates in moderate climates lies between 15° and 37°C. Some organisms are able to develop below, and others can survive above, these temperature limits. Certain organisms, largely found among the bacteria, fungi, actinomycetes, and algae, produce a normal growth and lead a natural existence at temperatures considerably in excess of the limits noted, namely at 50° to 65°C., and even at 75°C. They are usually designated as thermophilic forms. Among these, the bacteria have received the most attention, whereas the fungi and actinomycetes have been given little consideration. Most of the available information in regard to these organisms is limited to their occurrence in certain natural substrates and to their possible rôle in the self-heating of hay.

In the preparation of composts of stable manures and in the composting of plant residues, which give rise to the so-called artificial manures, the temperature of the compost is known to rise to 50–65° and may even reach 80°C. These high temperatures usually persist for many days. When the composts are turned, a drop in temperature takes place. This is rapidly followed by another rise, which in many instances does not reach the high point of the first. This process can be repeated three or four times, with gradually diminishing maximum temperatures. The rise of temperature in the compost is due to the active evolution of heat which accompanies the decomposition by microörganisms of the readily decomposable constituents in the plant residues. As long as there remains in the compost a considerable quantity of carbohydrates, namely, hemicelluloses and cellulose, the potentiality exists for a rise in temperature, under favorable conditions of moisture, aeration, reaction, and nitrogen supply. After the carbohydrate content has been markedly reduced as a result of the decomposition processes, the residual material may be considered to have become "humified." At this stage, the compost consists largely of lignins and their derivatives, of proteins, of mineral substances, and of varying amounts of residual carbohydrates and water-soluble compounds. Because of the limited capacity for rapid decomposition of this "humified"

[1] Journal Series paper, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

material, there is now little possibility for extensive temperature rises in the compost. If conditions in the original compost have been made unfavorable for rapid decomposition, as by the creation of anaerobic conditions, there is comparatively little rise in temperature.

Beginning with the early investigations (22, 3, 12) on the decomposition of plant residues by microörganisms in manures and composts and ending with some of the more recent studies (18, 29), it has become definitely established that heat evolution and rise in temperature are results of the activities of the microörganisms in the composts. It still remains to be determined whether a specific microbiological population is active at the different temperatures, whether one type of population is largely responsible for the rise in temperature, and whether this population is gradually replaced by other microörganisms as the temperature rises. There are two reasons for the lack of this specific information: 1. In plating out stable manure or compost material for determining the abundance of microörganisms, the plates are usually incubated at ordinary temperatures, ranging from 20° to 30°C.; under these conditions the specific thermophilic population either does not develop at all or is not recognized. 2. In those cases where thermophilic organisms have been found in the compost, little attempt has been made to correlate their occurrence with the chemical processes involved; the fact that an organism is found in the manure pile or in the compost heap is no evidence, as yet, that it is responsible for the particular condition of the compost.

In the following contribution, results of investigations on the thermophilic actinomycetes and fungus populations of soils and composts are reported. A study of population changes in composts with changes in temperature is reserved for another paper (26).

HISTORICAL

The existence of thermophilic bacteria in soil, feces, water, milk, and other natural substrates was first definitely established by Miquel in 1879 (13). His work was followed by numerous other investigations (21, 17, 10, 1, 9) on the occurrence of thermophilic organisms in various natural substrates. According to Mischustin (15), the abundance of thermophilic bacteria in soil can serve as an index of the cultivated state of the soil: cultivated soils, especially those receiving stable manures, are said to contain a larger number of these organisms.

Thermophilic actinomycetes, growing at 52–65°C., were first isolated from soil by Globig (5) in 1888. They were also isolated from manure by Rabinowitsch (20) and Tsiklinsky (24, 25); from soil by Gilbert (4); from hay by Miehe (12), Schütze (23), and Noack (17); from Sahara sand by Négre (16); from hay, feces, soil, air, and peat by Lieske (11); from human intestines by Bruini (2); and from sewage by Kedzoir (8).

It has been observed repeatedly that composted manure which has reached a high temperature or hay which has been allowed to heat in composts becomes covered with small white patches of fungus-like growth. Miehe (12) remarked that the appearance of these patches is similar to a coat of lime and is due to actinomycetes. Similar observations have been made by other investigators on thermophilic composts.

Tsiklinsky (24) inoculated potato with soil or with manure, and incubated it at 53–55°C. Isolations were made after 16 hours on agar plates incubated at 55–57°C. Two actinomycetes were obtained. One produced chains of spores and was, therefore, a true *Actinomyces*; the

other formed round or ovoid spores at the end of side branches, by the swelling of the tips. The mycelium and spores were easily stained with aniline dyes and the Gram stain, but ripe and detached spores were unstained except for a narrow border. The second organism was believed to be widely distributed in nature and was named *Thermoactinomyces vulgaris*. Because of its manner of spore formation, this form belongs to the group of actinomycetes later designated by Ørskov (19) as *Micromonospora*, and should, therefore, be classified as *Micromonospora vulgaris*. This organism grew at 48–68°C., but not at 70°, with an optimum at 57°C. It remained inert for a month at 37°C. or lower temperatures but became active, within 24 hours, at 56–57°C. The spores were said not to be destroyed at 100°C., even after 20 minutes. The organism resisted disinfectants well and grew readily on most ordinary media. A few of the colonies on the surface of the medium produced small white growths, which sometimes came together into a pellicle. The organism was proteolytic but not amylolytic. The *Actinomyces* (*Thermoactinomyces* II) was less weakly proteolytic, and the spores were less resistant to heat.

Gilbert (4) cultivated from various soil types several strains of a thermophilic actinomyces, which he designated as *Act. thermophilus*. Growth on potato was much folded, white, later becoming gray on the surface; the plug was darkened by some forms. The optimum temperature was 55°C.; growth ceased at 60°. Most strains ceased to grow at 45°C., whereas some could be adapted to grow on agar media at 37° and even at 22°C. The colonies on agar were, after 24–48 hours, small, folded, light yellow with a dark colored center. Gelatin was only slowly liquefied.

Miehe (12) considered the growth of actinomycetes to be characteristic of hot decomposing masses of plant material. Hot composts and not the soil were believed to be their natural substrates. The spores were found to lose their vitality rapidly, especially on agar media, but they survived on hay particles. One organism was designated as *Act. thermophilus* Berestnev; it grew best at 40–50°C., more slowly at 30°C., and not at all at 25° and 60°C. The manner of spore formation of this organism shows it to be a *Micromonospora*. Miehe reported, however, that he also saw thermophilic actinomycetes which formed spores according to the manner described by Gilbert. This suggests the probability that he had representatives of two different organisms. Schütze (23) found, in decomposing clover hay, representatives of these two thermophilic actinomycetes, one of which was designated as *Act. thermophilus* (Berestnev) and the other as *Act. monosporus* (Lehmann and Schütze); the latter was definitely a *Micromonospora*.

Noack (17) isolated from moist hay, kept at 45–46°C., a number of thermophilic fungi and actinomycetes. Various other thermophilic fungi were isolated from soil and from manure composts by a number of investigators. One of the most common of these fungi is a form belonging to the Fungi Imperfecti, isolated by Tsiklinsky and described as *Thermomyces lanuginosus*. It grew at 42–60°C., but not at 37° or lower temperatures. Miehe also isolated from hot composted hay a number of fungi including the *Thermomyces*. This group of organisms has been rather indefinitely described, and its exact relationship does not seem to be fully established. It belongs to those fungi which have been variously described under the generic names *Humicola*, *Monotospora*, *Sepedonium*, and *Acremonella*.

EXPERIMENTAL

Occurrence of thermophilic actinomycetes and fungi in soil

In none of the investigations reported in the foregoing section was any approach made to the study of the abundance, aside from occurrence, and of the functions of thermophilic organisms in soils or composts at normal or elevated temperatures. A study was, therefore, first made of the quantitative occurrence of thermophilic mocroörganisms in different soils, as influenced by

soil treatment. For this purpose a series of variously treated plots were selected from the experimental fields of the New Jersey Agricultural Experiment Station. These plots have been uniformly treated for the last 30 years, and marked changes have been established in the physical, chemical, and biological conditions of the soil. Samples were taken three times during the year; namely, in the winter (January) when the soil was frozen, in the spring (May), and in the summer (July). Several media were at first used, in order to determine the specific effect of the medium on the development of the organism. The plates were incubated at 50°C. for 2–5 days.

The results of analyses made on the field soils are presented in tables 1–3. They show that thermophilic actinomycetes are found in all soils at all seasons of the year. They were present abundantly, even in frozen soils, where they

TABLE 1

Influence of soil treatment on the thermophilic microbiological population of the soil—winter count (January, 1938)

Numbers per gram of moist soil

SOIL PLOT	SOIL TREATMENT	EGG-ALBUMEN AGAR		STARCH AGAR		NUTRIENT AGAR		ASPARAGINE-MANNITOL AGAR		AVERAGE PER CENT ACTINOMYCETES
		Bacteria	Actinomycetes	Bacteria	Actinomycetes	Bacteria	Actinomycetes	Bacteria	Actinomycetes	
7A	None	1,600	400	2,000	300	24,000	1,000	100	100	6.1
7B	Lime alone	4,000	700	1,700	300	57,000	500	381,000	2,000	1.1
19A	Minerals	1,200	500	3,100	700	15,700	200	200	700	9.4
19B	Minerals + lime	2,700	500	3,800	2,700	136,000	1,500	100,000	11,000	6.1
5A	Stable manure	4,000	2,000	25,000	21,000	178,000	7,500	109,000	4,500	10.3
5B	Manure + lime	94,000	20,000	15,000	13,000	52,000	500	450,000	58,000	13.0
18A	Stable manure + minerals	71,000	60,000	61,000	13,000	245,000	2,000	265,000	10,000	11.7
18B	Stable manure + minerals + lime	53,000	16,000	7,500	1,000	149,000	3,000	190,000	13,000	7.6

averaged 10,000–15,000 per gram; they were especially numerous in areas receiving liberal applications of stable manure. In the winter count, the proportion of thermophilic actinomycetes to thermophilic bacteria was somewhat higher in the plots receiving manure; in the spring count, however, there was very little difference. The most striking fact is that whereas, in the winter, the actinomycetes made up only a small part of the thermophilic population developing on the plates, in the spring count, they made up by far the major fraction of the thermophilic population.

The thermophilic fungi were not so numerous in the soil as were either the thermophilic bacteria or actinomycetes and hence were not recorded. Their numbers were usually less than 100 per gram of soil.

The obligate thermophilic nature of the actinomycetes and fungi was not so

pronounced as that of the bacteria. Whereas certain actinomycetes could grow at 50° and 65°C., they could also grow, with few exceptions, at 28°C. They might thus be considered as mesophilic forms with thermoduric properties, except for the fact that the ability to grow at 50° is not a property of any of the mesophilic forms. Over 100 cultures of mesophilic actinomycetes in our culture collection, which were previously isolated from soils, composts,

TABLE 2

Influence of soil treatment on the thermophilic population of the soil—spring count (May)

Numbers per gram of moist soil

SOIL PLOT*	EGG-ALBUMEN AGAR			STARCH AGAR		
	Bacteria	Actinomycetes	Per cent actinomycetes	Bacteria	Actinomycetes	Per cent actinomycetes
7A	400	3,200	88.8	4,000	10,900	73.1
7B	17,000	61,000	78.2	1,000	72,000	98.6
19B	6,000	67,000	91.7	3,000	43,000	93.4
5A	7,000	136,000	95.1	19,000	206,000	91.5
5B	25,000	140,000	85.3	22,000	280,000	92.7
18A	5,000	196,000	97.5	5,000	168,000	97.1
18B	5,000	95,000	95.0	18,000	175,000	90.6

* For treatment, see table 1; additions to manured plots made April 3, 1938.

TABLE 3

Influence of soil treatment on the thermophilic population of the soil—summer count (July)

Numbers per gram of fresh soil

SOIL PLOT	MOISTURE	EGG-ALBUMEN AGAR			STARCH AGAR		
		Bacteria	Actinomycetes	Per cent actinomycetes	Bacteria	Actinomycetes	Per cent actinomycetes
	per cent						
7A	8.8	200	3,600	94.7	200	400	66.7
7B	15.0	500	6,000	92.3	300	5,900	95.2
19A	13.4	2,000	29,000	93.5	5,000	5,000	50.0
19B	11.4	2,000	42,000	95.5	1,000	14,000	93.3
5A	16.8	5,000	447,000	98.9	5,000	135,000	96.4
5B	14.7	2,000	450,000	99.6	3,000	203,000	98.5
18A	15.0	12,000	393,000	97.0	8,000	315,000	97.5
18B	16.5	60,000	419,000	87.5	5,000	46,000	88.5

and other natural substrates, were tested. They not only did not grow at 50°, but were actually killed by exposure to this temperature, whereas the thermophilic forms were found to grow best at a temperature of 50°C. About half of the forms isolated grew vigorously also at 65°C.; no forms were observed growing much above this temperature, especially at or above 75°C. There was no apparent difference between the properties of the *Actinomyces* strains (I and

II) isolated from composts at 50°C. or 65°C. and those isolated from soils, even in a frozen condition.

The thermophilic fungi were found to grow at lower temperatures than the actinomycetes. Their division from the mesophilic forms was not so sharp. All grew at 28°C. and at 50°C., but none could grow, even in their natural environment, at 65°C.

In order to test the survival of thermophilic actinomycetes in soil and to determine the effect of manures upon the thermophilic population of the soil, the following experiment was performed. Sassafras sandy loam, of about pH 6.5, was placed in tumblers and treated with fresh manure, sterilized and unsterilized, with composted manure, and with pure cultures of thermophilic

TABLE 4

Influence of organic matter and of enrichment cultures on the number of thermophilic actinomycetes in soil kept at 28°C.

Numbers per gram of dry soil

TREATMENT	AT START		3 WEEKS OLD		5 WEEKS OLD	
	Bacteria	Actinomycetes	Bacteria	Actinomycetes	Bacteria	Actinomycetes
Control soil	172,000	93,000	10,000	7,200	2,500	4,800
Soil + fresh manure*	166,000	425,000	42,000	75,500	.....	.....
Soil + sterile manure†	93,000	275,000	22,000	24,500	.....	.....
Soil + thermophilic compost	74,000	564,000	640,000	1,550,000	500,000	1,250,000
Soil + sterile compost	97,000	628,000	12,000	8,000	3,000	3,000
Soil + thermophilic actinomycetes	172,000	68,500,000	103,000	2,880,000	.....	.....
Soil + sterile manure + thermophilic actinomycetes‡	172,000	20,200,000	141,000	431,000	.....	.....

* 2 gm. of fresh horse manure added to 100 gm. of soil.

† Manure sterilized by autoclaving for 15 minutes at 15 pounds' pressure.

‡ Quantity of inoculum used, smaller than that in foregoing treatment.

actinomycetes. The soil in the tumblers was adjusted to optimum moisture. The tumblers were covered and placed in a thermostat at 28°C. The soils were plated out immediately and after 3 and 5 weeks. Egg-albumen agar was used, and the plates were incubated at 50°C. The results presented in table 4 show that at 28° the thermophilic population of the soil, with and without the addition of manure, rapidly decreases. The only exception was found in the case of the thermophilic compost, where an actual increase of thermophilic actinomycetes occurred in the soil on incubation. No increase of the thermophilic actinomycetes occurred in the soil at the expense of the sterile compost. These results confirm the observations of Miehe concerning the survival in soil of thermophilic actinomycetes only in contact with the material upon which they developed.

Occurrence of thermophilic actinomycetes and fungi in thermophilic composts

The results of the previous experiment show definitely that thermophilic composts are an important source of thermophilic organisms in the soil. In order to study their development in the compost, fresh horse manure was placed in a series of glazed porcelain pots, and these were incubated at 50° and 65°C. The moisture was adjusted to 75–80 per cent. At different intervals, samples of the compost were plated out. The plates were incubated at the same temperature as the composts. The results (table 5) show that the highest number of thermophilic actinomycetes occurred in 10 days. The number per gram of moist compost kept at 50°C. was found to be 3,000,000,000 by the use of the nutrient agar plate, and 12,000,000,000 on the egg-albumen agar;

TABLE 5

Changes in numbers of fungi and actinomycetes in the process of decomposition of horse manure at moderately high temperatures

Millions per gram of moist compost

TEMPERATURE OF INCUBATION	PERIOD OF INCUBATION	NUTRIENT AGAR	EGG-ALBUMEN AGAR	
		Actinomycetes	Actinomycetes	Fungi
°C.	*days*			
50	1*	.....	30	...
50	2	75	1,000	...
50	6	650	5,000	...
50	10	3,000	12,000	200
50	15	2,500	2,000	...
50	20	2,200	2,000	20
65	1*	160	5	0
65	2	2,000	170	0
65	6	2,500	.....	0
65	10	9,000	.....	0
65	15	7,000	.....	0
65	20	3,000	.....	0

* 18 hours.

the 65°C. compost contained 9,000,000,000 actinomycetes, as determined by the nutrient agar plate.

The number of fungi reached 200,000,000 per gram of moist compost kept at 50°C. in 10 days and then began to diminish. There were no fungi on the plates prepared from the 65° compost. Bacteria were abundant in the composts kept at both temperatures.

These results show that thermophilic composts contain a highly characteristic microbiological population. In order to illustrate the activities of this population, the results of the proximate analysis of the manure decomposing at the two temperatures are given (table 6). Although the actual losses of the various chemical constituents have not been calculated, the percentage composition of the residual material indicates that, during the 10-day period,

decomposition of the manure was rapid at both temperatures but was somewhat more rapid at 65° than at 50°; this is shown by the relatively greater increase in ash, lignin, and protein at the higher temperature. On comparing these results with those reported previously (27) on the influence of temperature on the decomposition of plant residues, one finds a continuous increase in the rate of decomposition with an increase in temperature, from 0° to 65°C. The 65° maximum seems to take place, however, only during the first few days of decomposition. It will be shown in another contribution (26) that, after the first few days, the 50° compost becomes more active than the 65° compost.

In the above experiment, slides were placed in close contact with the manure and removed at various intervals during the early stages of decomposition of the composts. The slides were stained with phenolic rose bengal for 12–15 minutes over a steam bath. They were examined with a compensating microscope using various combinations of oculars and objectives, the higher power being a 2-mm. apochromatic objective (N.A. 1.30) and 12.5× oculars.

TABLE 6

*Influence of temperature on the decomposition of horse manure**

Per cent of dry material

TEMPERATURE OF INCUBATION	WATER-SOLUBLE ORGANIC MATTER	HEMICELLULOSES	CELLULOSE	LIGNIN	ASH	PROTEIN
°C.						
Fresh manure	4.5	20.6	30.9	20.5	8.9	5.8
50	5.8	14.6	18.9	24.0	12.4	12.6
65	6.3	14.2	20.1	26.2	16.8	13.1

* Incubation 10 days.

A Bausch and Lomb type H camera, tungsten filament lamp, and green filter were used for making photographs, none of which has been retouched. The results obtained are presented in plates 1, 2, and 3. It is, of course, to be remembered that the microbiological population thus obtained is highly localized, being limited to the contact area of the slide.

Although various fungi have been reported (24, 12) to be thermophilic in nature and to occur in thermophilic composts, the predominating fungus found in these composts of horse manure was the organism designated by Tsiklinsky and Miehe as *Thermomyces*. It was found in great abundance in the 50° compost, but not at 65°C. Once isolated from the compost, it could also grow, though much more slowly, in pure culture at lower temperatures such as 28°C. This organism was found capable of decomposing virtually all the organic constituents of the composts and to behave, in this respect, in a manner similar to the whole thermophilic population, as shown later. It can attack hemicelluloses, cellulose, and even lignin, the last compound to a much smaller extent than the first two.

The course of development of this fungus on the 50°C. compost and its relation to the rest of the compost population are illustrated in plate 1. Within 18 hours, the whole compost is found to be permeated with the mycelium of the fungus (fig. 1). A somewhat higher magnification of an isolated clump of mycelium reveals extensive radiation of the multicellular hyphae from a single center, which probably was a spore or a group of spores of the fungus in question (fig. 2). Within 2 days, the mycelium is found to be covered with a great number of black chlamydospores, arising on short side branches or within the mycelium (fig. 3). When the mass of growth is magnified, the chlamydospores are found to be of various sizes. The mass of fungus growth was found to be accompanied, within 2 days, by bacterial masses, occurring either as individual cells or as localized colonies (fig. 4). Within 6 days, the mycelium rapidly disintegrated, and its place was taken by large numbers of bacteria occurring all along the mycelial threads, as chains, as individual cells, or as zoogleal masses (fig. 5). In addition to the fungus and bacterial populations which characterize the 50° compost, there was also an extensive population of actinomycetes, which almost completely replaced, within a very few days, the fungus population of the 50° compost (fig. 6). At 65°C., the fungi did not develop at all; the bacteria were the first organisms to develop, and this development was soon accompanied by extensive growth of actinomycetes.

The thermophilic actinomycetes developing in the composts of horse manure are illustrated in plates 2 and 3. This population can be very definitely divided into two distinct taxonomic groups, namely, the genus *Actinomyces* (plate 2) and the genus *Micromonospora* (plate 3). Each of these genera was represented in the thermophilic composts by at least three species, easily recognizable in the stained preparations because of their specific morphological characteristics:

Actinomyces I, with straight aerial mycelium, readily breaking up into spores.
Actinomyces II, with spiral-forming aerial mycelium.
Actinomyces III, with chains of spores arising in the form of side branches directly on the mycelium.
Micromonospora I, with single spores on the tips of side branches of the mycelium; these spores can be very close to the mycelium, or on short side branches, or on longer side branches.
Micromonospora II, in which the spore-bearing hyphae are branching.
Micromonospora III, with spores borne in the form of clusters.

The first two species of the genus *Actinomyces* are similar to many other forms commonly found in soils and in other natural substrates, and were isolated many times from soils and from composts in our own investigations. The three species of the genus *Micromonospora*, however, seem to be more representative of thermophilic composts, where they have been found by Tsiklinsky, Miehe, and Schütze, although they have also been found in normal soils by Jensen (6, 7).

Actinomyces I, shown in figures 7 and 8, was found extensively in the 50° and 65°C. composts. *Actinomyces* II is shown in figure 9. *Actinomyces* III,

shown in figures 10, 11, and 12, was found in the 50° and 65° composts. The first two organisms were readily isolated in culture, but the third form has so far been observed only in microscopic preparations.

Micromonospora I was found extensively in the 50° compost, as shown by figures 13, 14, and 15. Figure 14 shows the *Micromonospora* accompanied by a *Thermomyces* which brings out the relative sizes of the fungus and the actinomycete.

Micromonospora II is shown in figure 16; it was found in the 6-day-old 65° compost. *Micromonospora* III is shown in figures 17 and 18. It was found in the 1-day and 15-day-old composts at 50°C.

Many types of bacteria were present in both composts. Some of them were spore-forming, especially in the higher temperature composts (fig. 8). Their relation to the thermophilic fungi and actinomycetes is brought out in another paper (26), where further information is given concerning the occurrence of thermophilic actinomycetes and fungi in manure composts.

It is of interest to emphasize the results of the cultural or plate studies and of the microscopic studies of the thermophilic population of composts. Both methods brought out the presence and course of development of the fungi and their rôle in the sequence of populations in these composts. Of the actinomycetes, only two species of *Actinomyces* were commonly found on the plate and could easily be isolated, and the *Micromonospora* could be isolated only by means of enrichment cultures. Under the microscope, however, the great abundance of *Micromonospora* in the composts could hardly be overlooked.

The results of these investigations thus emphasize again the fact that the microscopic methods for studying the populations of soils and composts can bring out the presence and development of organisms either not readily grown on the plate or occurring in a state which is not differentiated by the plate method, namely, as mycelium vs. spores. The plate method, however, enables the isolation and cultivation of the specific organisms, for a study of their biochemical activities and for a determination of their specific rôle in the transformation processes taking place in the composts. The two methods can, therefore, readily supplement each other.

Activities of thermophilic fungi and actinomycetes

The functions of thermophilic fungi and actinomycetes at normal or mesophilic temperatures is a matter for further study, although it is doubtful whether these two groups of thermophilic organisms play any significant part at those temperatures. Lieske reported (11) that thermophilic actinomycetes were found to survive for many months in pure culture on artificial media, at lower temperatures, namely, at 28° and even at 0–5°C. At these temperatures, they were unable to compete with a mixed mesophilic population and gradually died off when introduced into soils. The same was true of our own cultures. The presence of these organisms in a variety of soils seems to be related to their ability to survive under mesophilic conditions rather than to

any significant rôle which they might play at these temperatures. More attention was therefore given to composts in which the rôle of the thermophilic forms is much more significant.

In order to throw some light on the specific functions of the various organisms concerned in the decomposition of stable manure at moderately high temperatures, an experiment was conducted whereby representative pure cultures of thermophilic bacteria, fungi, and actinomycetes were inoculated into sterile horse manure and their activities compared with those of a mixed population found in fresh unsterilized manure. Incubation took place at 50°C., for 42 days. The results (table 7) show very definitely that neither the bacteria nor the actinomycetes could bring about, in pure culture, as much decomposition of the manure as a whole or of its specific constituents, as did the mixed population. The fungus was the only organism that approached the total population

TABLE 7

*Decomposition of stable manure by pure cultures of thermophilic microorganisms and by a mixed thermophilic population**

Per cent of dry material

INOCULUM	TOTAL DRY MATERIAL	TOTAL NITROGEN	WATER-SOLUBLE ORGANIC MATTER	HEMICELLULOSES	CELLULOSE	LIGNIN	PROTEIN	ASH
Manure control	100.0	2.32	9.8	22.8	19.7	18.5	11.0	9.8
Bacterium 1	75.3	2.52	10.2	18.9	17.3	24.0	13.4	11.3
Bacterium 2	85.1	2.34	6.3	19.9	20.6	22.1	13.1	10.4
Thermophilic Actinomyces (white) (I)	76.4	2.53	11.9	17.9	17.9	21.0	12.6	12.7
Thermophilic Actinomyces (brown) (II)	89.6	2.27	8.3	18.1	19.0	19.9	11.1	11.6
Thermophilic fungus	60.7	2.98	18.6	17.2	12.6	23.6	13.6	14.4
Natural population of manure	37.9	4.07	16.3	11.7	4.6	18.4	19.9	24.2

* Incubation for 42 days at 50°C.

in the extent of decomposition. It decomposed 40 per cent of the total manure, as compared with 62 per cent for the mixed population. The fungus brought about the destruction of a greater than proportional amount of the hemicelluloses and cellulose, but it attacked the lignin only to a limited extent, as compared with the mixed population. The increase in water-soluble material was greater in the case of the fungus and the synthesis of protein was less; this was to be expected because of the more limited decomposition of the carbohydrates. The bacteria and actinomycetes grew largely at the expense of the hemicelluloses and the water-soluble constituents. These results tend to emphasize the fact that in the decomposition of manure at moderately high temperatures, a variety of microörganisms have a variety of specific functions. No single organism, no matter how active, can compare, in the rapidity of decomposition of the manure, with a mixed population. The fact that certain organisms,

notably actinomycetes, may be much more active in breaking down plant constituents in mixed populations than in pure culture has been amply demonstrated elsewhere (28).

Classification and description of thermophilic actinomycetes

With the recognition of the universal distribution of actinomycetes in nature, as animal and plant pathogens and as saprophytes occurring on a great many natural substrates, with the introduction of synthetic media for the growth of these organisms, and with increasing knowledge concerning their varying morphology, need was felt for the further separation of these organisms into several generic groups. Most of the descriptions of these organisms are based on their occurrence in nature, or their relation to oxygen and to heat, on pigment formation, or on growth characters upon artificial culture media. This information, however satisfactory it may be for species differentiation, was hardly sufficient for the separation of the group into several genera. Ørskov (19) deserves the credit for having made the first systematic attempt at such a classification. He divided the actinomycetes into three groups, as follows:

I. *Cohnistreptothrix*, in which the substratum mycelium shows no spontaneous division and the somewhat thicker aerial mycelium divides into spores.

II. *Actinomyces*, in which both the substratum and the aerial mycelium divide spontaneously into spores.

III. *Micromonospora*, which forms single oval spores at the tips of the branching mycelium.

Jensen (6, 7) recognized only two genera in the family *Actinomycetaceae;* namely, *Actinomyces*, comprising all actinomycetes which form chains of spores, and *Micromonospora*, which forms single terminal spores. This system, which is the simpler one, may be accepted for the purpose of the following discussion.

Both *Actinomyces* and *Micromonospora* were found abundantly in soils and in composts, with one important difference, namely, that the genus *Actinomyces* is much more predominant in soils, whereas *Micromonospora* seems to be very abundant in hot composts. It is of particular interest, in connection with this investigation, to direct attention to the occurrence of the latter group. Miquel (14), in his pioneer study of the microörganisms of the atmosphere, published in 1883, reports that side by side with ordinary bacterial filaments, the dust of the atmosphere carries also branching bacteria. His descriptions and illustrations leave no doubt that he had a respresentative of the *Micromonospora* group. As to the organism commonly known in the literature as *Act. thermophilus* Berestnev, it is difficult to say whether the same form was always had by those investigators who claimed to have isolated it. An attempt will be made to define this form more definitely. The organism isolated by Tsiklinsky, in 1899, from hot composts is definitely a member of this group. Isolations of similar organisms were made by Miehe from hot hay.

Actinomyces species.—Two of the three species of *Actinomyces* (I and II) described here were readily isolated in culture. They were found to be very abundant in soils and in composts and grew at both 50° and 65°C. They possessed a striking morphological and physiological constancy. They differed primarily in their pigment formation on sucrose-nitrate (Czapek's) agar, one remaining white and the other becoming gray-brown. Both forms produced abundant aerial mycelium with characteristic spore formations. The white form produced its spores by the regular division of the uncurled, or straight, nonspiral-forming mycelium. In the gray-brown form, spores were produced by means of characteristic spirals, typical of many soil actinomycetes. Physiologically the two species were very similar, both actively hydrolyzing starch, gelatin, milk; they grew well on cellulose, on straw, on potatoes, and on the common bacteriological media. They acted on sugars, giving no acid or gas, and were aerobic in nature. Their characteristic properties were those of mesophilic forms of the *Act. albus* group. About half of the strains within each species grew well at 65°, the other half being somewhat inhibited at this temperature. At 50°C., however, there was no detectable difference between the various strains. It remains, therefore, for future study to determine whether or not growth at 65°C. may be used as a basis of further subdivision of the thermophilic actinomycetes. So great was the apparent predominance of these two types of *Actinomyces* in composts and in soils, that it was difficult to demonstrate culturally the presence of other types of actinomycetes.

Table 8 gives a summary of the characteristics of the various strains of the two species of thermophilic *Actinomyces* isolated from the variously treated soils and composts. On the basis of these observations, the following descriptions are presented here:

Act. thermophilus (Berestnev) was isolated from soils, hay, and composts by Globig, Gilbert, possibly Miehe and Schütze, and ourselves. It grows best at 50°C., grows also at lower temperatures (28°C.), but seldom grows at 60°. Some strains were isolated which were not capable of growing at 28°C., whereas others seem to grow well even at 65°. It produces, at 28°C., a deep colorless growth on Czapek's agar, thin white aerial mycelium, no soluble pigment; a yellowish growth on potato plug, with no aerial mycelium, the plug usually being colored brown; no pigment on nutrient agar or gelatin; yellowish growth on starch agar, with white-gray, powdery aerial mycelium. Same type of growth at 50°, but more abundant. The organism is proteolytic, amylolytic, and forms straight sporulating hyphae.

Act. thermofuscus n. sp. is distinguished from the foregoing species by the brown-colored aerial mycelium on synthetic media, spiral-shaped spore-bearing hyphae, and ability to grow readily at 65°C. It grows faintly at 28°, producing a thin, deep-gray growth on Czapek's agar, with but little aerial mycelium. Growth is better, at 28°, on nutrient agar and on gelatin, which is liquefied, without the formation of any soluble pigment. At 50°, the growth on Czapek's is dark to violet, with gray to lavender aerial mycelium and soluble brown pigment; abundant, dark-colored growth on potato, no aerial mycelium, or few white patches, with dark soluble pigment. On gelatin, a grayish ring is produced, the gelatin is liquefied, and soluble pigment is formed. At 60°, growth is similar to that of 50°.

The course of development of these two *Actinomyces* species is shown in plate 4. During the early stages of growth (fig. 19, 20), the two species are

TABLE 8

Summary of morphological and physiological characters of various strains of thermophilic actinomycetes isolated from soils and composts

STRAIN	SOURCE	GROWTH			MORPHOLOGY OF AERIAL MYCELIUM ON CZAPEK'S AGAR	SYNTHETIC AGAR		POTATO-SLANT			STARCH AGAR		GELATIN		
		28°	50°	65°		Growth	Color*	Growth	Color* of aerial mycelium	Color* of plug	Color* of growth	Hydrolysis of starch	Growth	Color*	Liquefaction
1†	Soil 4A	+	+	−	Straight	±	W	±	W	Br	W	++	++++	W	+
2†	Soil OA, 12A, 8A	+	+	+	Straight	±	W	−	....	....	W	+++	++	S-Y	+
3†	Soil OA, 12A, 8A	+	+	+	Straight	±	W	−	....	....	W	+++	++	S-Y	±
4‡	Soil 6A	±	+	+	Spiral	++	Gr-Br	++	W	Br	W	+++	±	S-Y	+
5†	Soil 13A	+	+	−	Straight	±	W	±	W	Br	W	+++	±	S-Y	±
6‡	Soil 4A	+	+	+	Spiral	±	Gr-Br	++	Br	Br	W	+++	+++	Gr	++
7†	Soil 5AA, 7AS	−	+	+	Straight	±	W	++	W	Br	W	++	+++	W	++
8‡	Soil 5B, 19B, 18B	+	+	−	Spiral	++	Gr-Br	+++	W	Br	W	+	+++	Gr	±
9‡	Soil 5B, 5A, 19A, 18A	+	+	±	Spiral	++	Gr-Br	+++	Gr	Br	W	+	+++	W	++
10‡	Soil 5B, 18B	+	+	−	Spiral	++	Gr-Br	+++	Gr	....	W	+	+++	W	++
11‡	Soil 19A	+	+	+	Spiral	+	Gr-Br	++	Br	....	W	+++	+++	W	++
12†	Soil 5A, 19A, 18B, 5B	+	+	±	Straight	±	W	++	W	....	W	+++	+++	W	+
13‡	Soil 19A	+	+	±	Spiral	++	Gr-Br	+	W	Br	W	+	+++	W	++
14†	Soil 5A, 19A, 5B	−	+	−	Straight	+	W	−	....	....	W	+++	+++	W	++
15†	Compost 65°	+	+	+	Straight	+	W	+	W	....	W	++	++	W	++
16‡	Compost 65°	+	+	+	Spiral	±	Gr-Br	+	Gr	....	W	+	++	Gr	++
17‡	Compost 65°	+	+	+	Spiral	+	Gr-Br	+	W	....	W	+++	+	W	++
18†	Compost 65°	+	+	+	Straight	+	W	+	Gr	....	W	+++	+	W	+
19†	Compost 65°	+	+	+	Straight	+	W	+	W	....	W	+	++	W	+
20†	Compost 50°	+	+	−	Straight	+	W	±	W	....	W	+	+++	W	+
21‡	Compost 50°	±	+	+	Spiral	++	Gr-Br	+++	Gr	Br	W	+	+++	Gr	±

* W = white; Gr = gray; Br = brown; S-Y = submerged growth-faint yellow; Gr-Br = gray-brown.

† *Act. thermophilus.*

‡ *Act. thermofuscus.*

undistinguishable. Their mode of growth and spore formation are comparable to those of the mesophilic forms. When spore formation begins, one is able to differentiate easily between the *Act. thermophilus* type and the *Act. thermofuscus* type. The former bears its spores on straight strands of aerial hyphae (early spore formation is shown in fig. 21); this process extends gradually along the hyphae until their whole length becomes sporulated (fig. 22). The latter organism produces typical sporulating spirals (fig. 23).

Actinomyces III is distinctly different from the other two species, because of the manner of spore formation. The only other organism of a similar nature was a thermophilic *Actinomyces* isolated by Lieske (No. 89) from animal excreta. This organism bears a close resemblance to *Act. reticuli* Waksman and Curtis and to *Act. reticulus-ruber* Waksman, which produce the sporogenous hyphae in knotlike groups along the central mycelium or along branches of this mycelium. These organisms form a special group, distinct from the genera *Actinomyces* and *Micromonospora*.

Micromonospora species.—Species of *Micromonospora* observed on the contact slide resembled closely the organisms of Tsiklinsky, Miehe, and Schütze. The three species of *Micromonospora* found in composts were similar to the mesophilic forms described by Jensen (6, 7). Only one of them has been isolated in pure culture; the other two, observed abundantly in both the 50° and 65°C. composts, have not as yet been isolated. The formation of new species, based on the thermophilic nature of the organisms, is not justified before important physiological or morphological differences have been established.

Micromonospora I is similar to *Micr. coerulea* of Jensen. It is also similar to the form previously named by Tsiklinsky. The suggested designation *Micr. vulgaris*, therefore, deserves priority. The *Act. monosporus* of Schütze definitely belongs to this group. Other thermophilic *Micromonospora* of type I have been isolated by Miehe and mentioned by Lieske.

Micromonospora II falls definitely within the group *Micr. chalceae* (Foulerton) described in detail by Ørskov and by Jensen.

Micromonospora III represents a large group of thermophilic actinomycetes, found extensively in 65°C. composts. *Micr. fusca* Jensen may be considered as representative of this group.

Five strains of *Micromonospora vulgaris* [*Micr. vulgaris* (Tsiklinsky) n. des., syn. *Micr. coerulea* Jensen] have been isolated by means of the potato plate method of Tsiklinsky (one from the 65°C. compost, one from 50° compost, and three from a naturally heating compost). These five strains are identical and correspond even in minor details to the description given by Tsiklinsky (24). Culturally, the organisms grow well on beef-peptone agar, on potato, milk, beef-peptone broth, etc. On Czapek's agar, the growth is white, powdery, slightly raised. The organism produces no turbidity in broth, but only a tough white pellicle and in many instances a considerable number of ball-like colonies at the bottom of the tube. On other media, a white, powdery, thin

aerial mycelium is produced which is hardly raised above the surface. No pigment is formed. The strains liquefy gelatin, coagulate and gradually liquefy milk, but (as reported by Tsiklinsky) do not hydrolyze starch, though faint traces of hydrolysis may be noted after 6 days. The isolated strains grow better at 60° than at 65°C.

Morphologically the development of these organisms is entirely comparable to that of the mesophilic form described by Jensen (6, 7). The young mycelium shows slightly more branching than that produced by *Actinomyces* species (fig. 24, which also shows germinating spore). Spores are borne at the end of short branches (fig. 25) from which they are easily broken. Figure 26 shows a late stage of spore formation. The aerial mycelium, though present, is usually rudimentary, rarely exhibiting the tangled network of strands typical of *Actinomyces* species. These strains of *Micromonospora vulgaris* differ thus from the mesophilic forms, which show no trace of aerial mycelium. Fragmentation has not been seen in slide cultures of the organisms thus far isolated, but it was found to occur in smear preparations, and fragmentation of the mycelium was noted on many of the contact slides.

SUMMARY

Thermophilic actinomycetes were found abundantly in variously treated field soils, those receiving stable manure containing larger numbers of these organisms. Although they grew at 50° and 65°C., most of them could also grow readily at 28°. They were, therefore, not obligate but facultative thermophiles.

Thermophilic actinomycetes grown on agar media and added in suspension to soils, kept at room temperature, died out rapidly. When these organisms were introduced with thermophilic composts, they survived readily in the soil. There was no multiplication of the thermophilic actinomycetes in soil kept at 28°C.

Composts of horse manure kept at 50° and 65°C. developed an extensive and highly characteristic population of thermophilic fungi and actinomycetes.

Among the fungi, the most abundant organism belongs to the ill-defined group described as *Thermomyces*. This is a member of the Fungi Imperfecti and produces dark chlamydospores on short side branches. It grew very extensively at 50° but not at 65°C. It compared favorably, in its ability to decompose the organic constituents of the manure, with the total thermophilic population of the compost.

The fungi seemed to play an important part in the sequence of populations in the 50°C. compost. They appeared within 18 hours, in the form of an extensive mycelium. They soon sporulated and were followed by bacteria, many of which seemed to grow largely at the expense of the fungus mycelium.

Among the thermophilic actinomycetes, six distinct types were recognized, belonging to two genera, namely, *Actinomyces* and *Micromonospora*.

Two *Actinomyces* were constantly isolated in pure culture; they were found extensively in both 50° and 65°C. composts, as well as in soils.

Only one of the three species of *Micromonospora* could be readily isolated in culture. They could all, however, be identified, from microscopic preparations, with organisms isolated by other investigators from air, soil, and composts.

Pure cultures of thermophilic microörganisms, especially bacteria and actinomycetes, were found to be less active than the mixed population in breaking down the constituents of composts. Only the thermophilic fungi could compare, in their action, with the mixed population. This is due to the greater selective action of bacteria and actinomycetes upon the various specific constituents of the manure and to the interrelationships of the mixed population.

REFERENCES

(1) Bergey, D. H. 1919 Thermophilic bacteria. *Jour. Bact.* 4: 301–306.
(2) Bruini, G. 1905 Über die thermophile Mikrobenflora des menschlichen Darmkanals. *Centbl. Bakt.* (I) 38: 177–185, 298–307.
(3) Dupont, C. 1902 Sur les fermentations aerobies du fumier de ferme. *Ann. Agron.* 28: 289–317.
(4) Gilbert, Dr. 1904 Über *Actinomyces thermophilus* und andere Actinomyceten. *Ztschr. Hyg. u. Infectionskrank.* 47: 383–406.
(5) Globig 1888 Über Bakterienwachstum bei 50 bis 70°C. *Ztschr. Hyg. u. Infectionskrank.* 3: 294–321.
(6) Jensen, H. L. 1930 The genus *Micromonospora* Ørskov, a little known group of soil microörganisms. *Proc. Linn. Soc. N. S. Wales* 40: 231–248.
(7) Jensen, H. L. 1932 Contributions to our knowledge of the actinomycetales. III. Further observations on the genus *Micromonospora*. *Proc. Linn. Soc. N. S. Wales* 42: 173–180.
(8) Kedzoir, D. 1896 Über eine thermophile *Cladothrix*. *Arch. Hyg.* 27: 328–338.
(9) Krohn, V. 1923 Studien über thermophile Schizomyceten. *Ann. Acad. Sci. Fennica* (A) 21: 125.
(10) Kroulik, A. 1913 Über thermophile Zellulosevergärer. *Centbl. Bakt.* (II) 36: 339–346.
(11) Lieske, R. 1921 Morphologie und Biologie der Strahlenpilze. Leipzig.
(12) Miehe, H. 1907 Die Selbsterhitzung des Heues. G. Fischer, Jena.
(13) Miquel, P. 1881, 1888 Bulletin de la statistique municipale de la ville de Paris. Dec. 1879. [Cited by Tsiklinsky (24) and Miehe (12)]; *Ann. Microg.* 1: 4–10.
(14) Miquel, P. 1883 Les Organisms Vivants de l'Atmosphére. Paris.
(15) Mischustin, E. N. 1935 Thermophilic bacteria as an indicator of state of soil cultivation. *Microb. Lab. VIUAA., U. S. S. R.*, p. 55–80.
(16) Négre, L. 1913 Bactéries thermophiles des sables du Sahara. *Compt. Rend. Soc. Biol.* [Paris] 74: 814–816.
(17) Noack, K. 1912 Beiträge zur Biologie der thermophilen Organismen. *Jahrb. Wiss. Bot.* 51: 593–648.
(18) Norman, A. G. 1930 The biological decomposition of plant materials. III. Physiological studies on some cellulose-decomposing fungi. *Ann. Appl. Biol.* 17: 575–613.

(19) ØRSKOV, J. 1923 Investigations into the Morphology of the Ray Fungi. Copenhagen.
(20) RABINOWITSCH, L. 1895 Über die thermophilen Bakterien. *Ztschr. Hyg. u. Infectionskrank.* 20: 154–164.
(21) SAMES, T. 1900 Zur Kenntnis der bei höherer Temperaturen wachsenden Bakterien und Streptothrixarten. *Ztschr. Hyg. u. Infectionskrank.* 33: 313–362.
(22) SCHLÖSING, T., AND SCHLÖSING, T., JR. 1892 Contribution à l'étude des fermentations du fumier. *Ann. Agron.* 18: 5–18.
(23) SCHÜTZE, H. 1908 Beiträge zur Kenntnis der thermophilen Aktinomyzeten und ihrer Sporenbildung. *Arch. Hyg.* 67: 35–56.
(24) TSIKLINSKY, P. 1899 Sur les mucedinées thermophiles. *Ann. Inst. Pasteur* 13: 500–505.
(25) TSIKLINSKY, P. 1903 Sur la flore microbienne thermophile du canal intestinal de l'homme. *Ann. Inst. Pasteur* 17: 217–240.
(26) WAKSMAN, S. A., CORDON, T. C., AND HULPOI, N. Influence of temperature upon the microbiological population and decomposition processes in composts of stable manure and plant residues. *Soil. Sci.* (to be published).
(27) WAKSMAN, S. A., AND GERRETSEN, F. C. 1931 Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microorganisms. *Ecology* 12: 33–60.
(28) WAKSMAN, S. A., AND HUTCHINGS, I. J. 1937 Associative and antagonistic effects of microorganisms: III. Associative and antagonistic relationships in the decomposition of plant residues. *Soil Sci.* 43: 77–91.
(29) WAKSMAN, S. A., AND NISSEN, W. 1932 On the nutrition of the cultivated mushroom, *Agaricus campestris*, and the chemical changes brought about by this organism in the manure compost. *Amer. Jour. Bot.* 19: 514–537.

PLATE 1. THERMOPHILIC FUNGI IN COMPOSTS OF HORSE MANURE

FIG. 1. *Thermomyces* in 50°C. compost, 18 hours old. × 102.

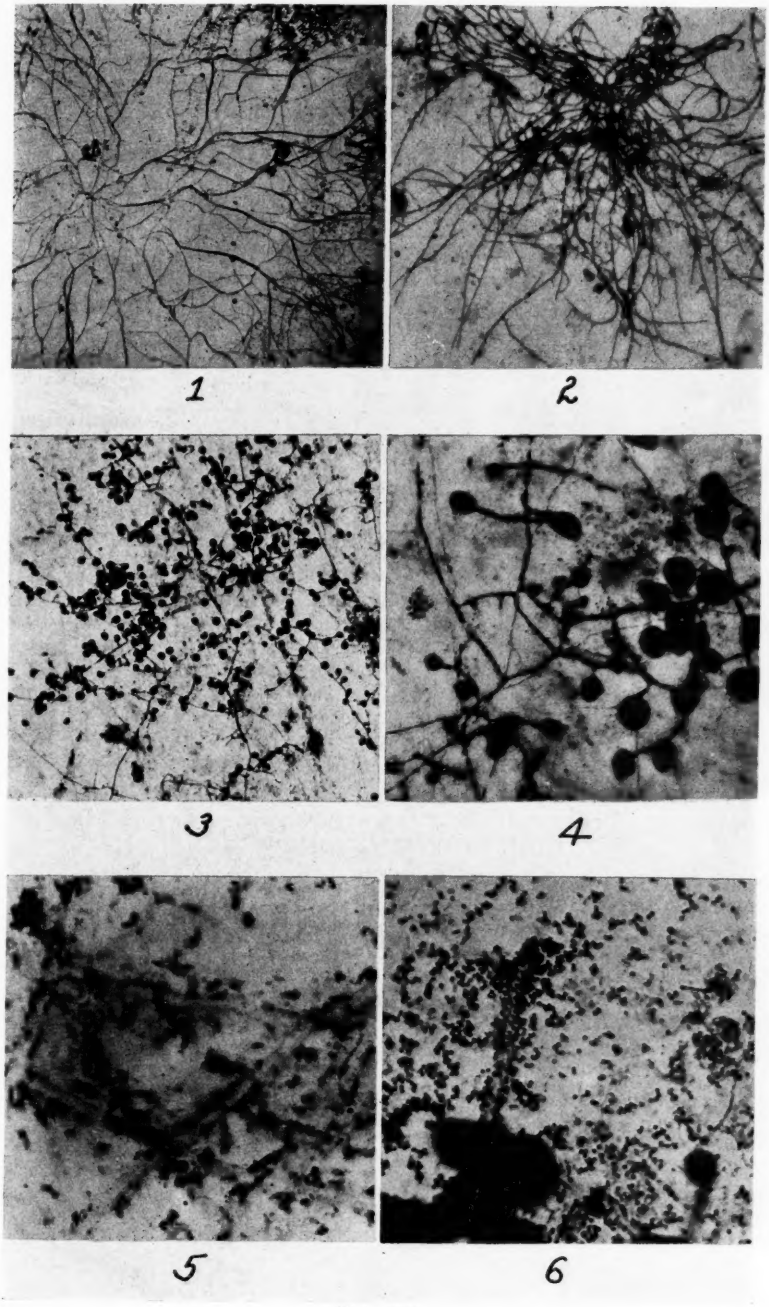
FIG. 2. *Thermomyces* mycelium in young compost at 50°. × 446.

FIG. 3. Actively sporulating *Thermomyces* in 50° compost, 48 hours old. × 102.

FIG. 4. Details of mycelium and spore formation by *Thermomyces*, developing in 48-hour compost. Note also bacterial masses. × 446.

FIG. 5. Rapid destruction of fungus mycelium by bacteria in 6-day-old compost. × 956.

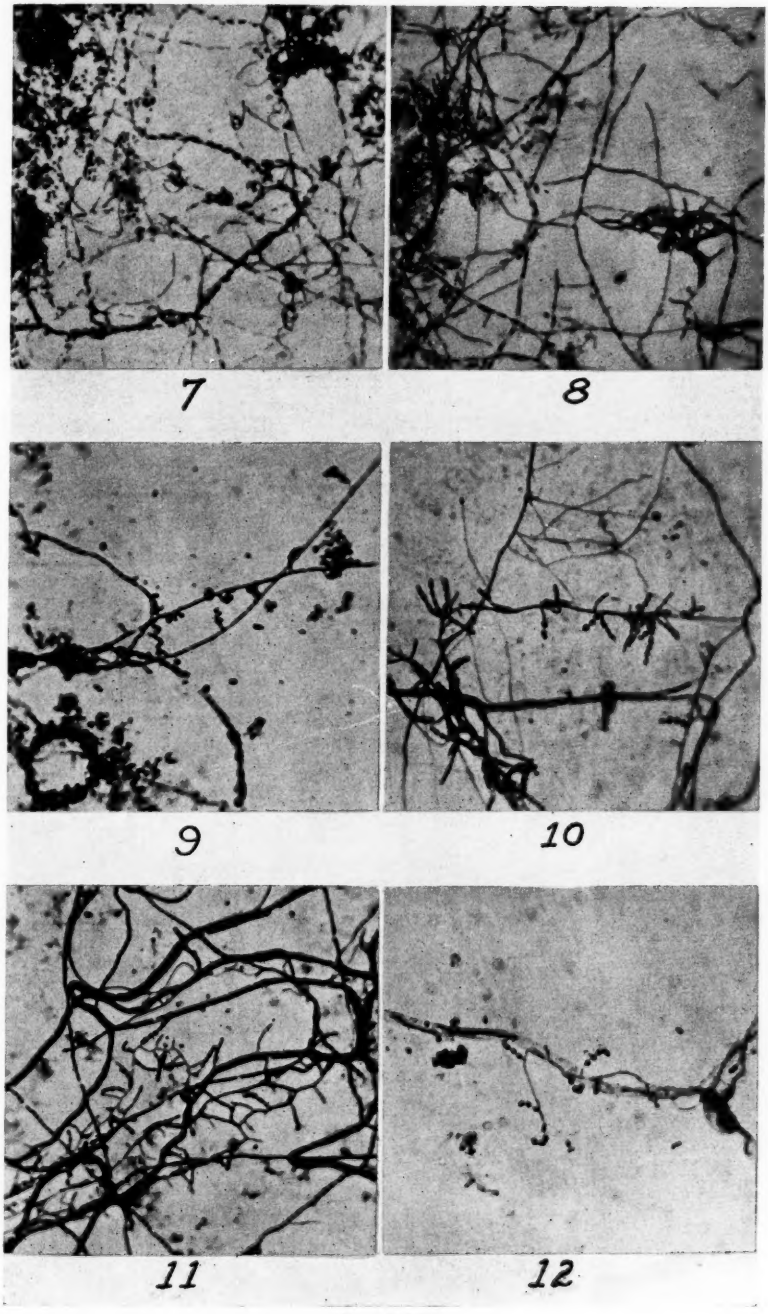
FIG. 6. Masses of fungus spores, bacteria, actinomycetes spores on remnants of plant material in 15 days. × 971.



FIGS. 1–6

PLATE 2. THERMOPHILIC SPECIES OF ACTINOMYCES IN COMPOSTS OF HORSE MANURE

FIG. 7. *Act. thermophilus* in 50°C. compost, 48 hours old. × 956.
FIG. 8. *Act. thermophilus* accompanied by bacteria in 65°C. compost, 48 hours old. × 956.
FIG. 9. *Act. thermofuscus* in 50°C. compost, 19 days old. × 956.
FIG. 10. *Actinomyces* III in 65°C. compost, 10 days old. × 956.
FIG. 11. *Actinomyces* III in 65°C. compost, 10 days old. × 956.
FIG. 12. *Actinomyces* III in 50°C. compost, 15 days old, accompanying fungus mycelium. × 956.



FIGS. 7–12

PLATE 3. THERMOPHILIC SPECIES OF MICROMONOSPORA IN COMPOSTS OF HORSE MANURE

FIG. 13. *Micr. vulgaris* in 65°C. compost, 15 days old, showing spores close to mycelium. × 956.

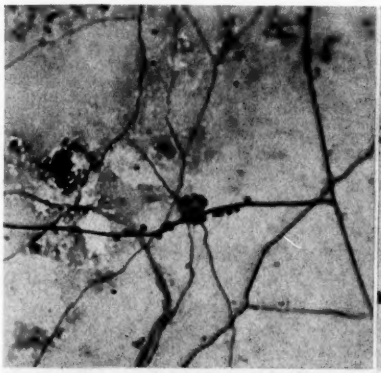
FIG. 14. *Micr. vulgaris* on short side branches, together with sporulating Thermomyces in 50°C. compost, 2 days old. × 956.

FIG. 15. *Micr. vulgaris*, showing extensive spore formation on short side branches, in 50° compost, 2 days old. × 1275.

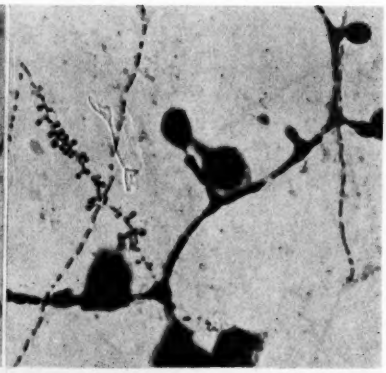
FIG. 16. *Micr. chalceae* in 65°C. compost, 6 days old. × 956.

FIG. 17. *Micr. fusca* in 50°C. compost, 1 day old. × 956.

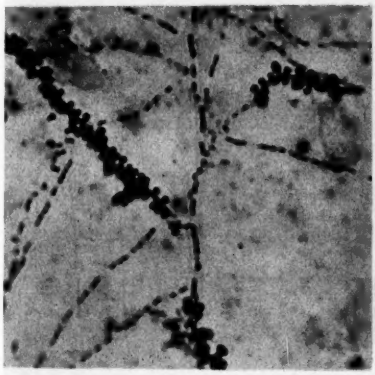
FIG. 18. *Micr. fusca* in 50°C. compost, 15 days old. × 956.



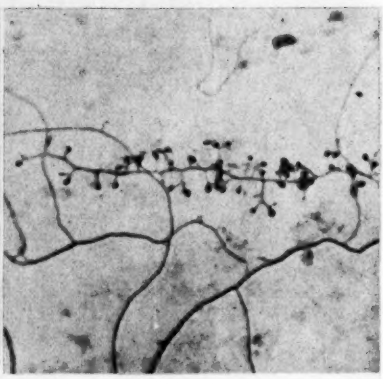
13



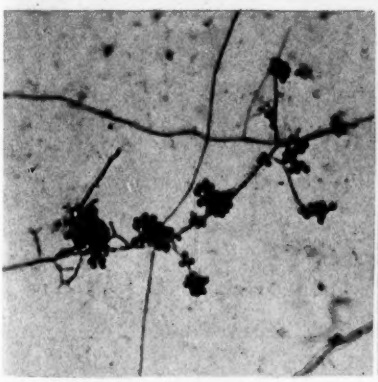
14



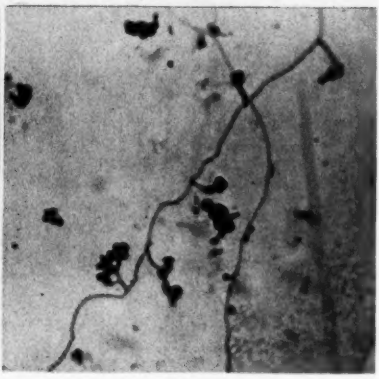
15



16



17



18

FIGS. 13–18

PLATE 4. PURE CULTURES OF ACTINOMYCETES

FIG. 19. *Act. thermophilus*. Gram stain of a 6-hour slide culture (50°C.) showing germinating spores and initial mycelium development. × 937.

FIG. 20. *Act. thermophilus*. Aerial mycelium just emerging from substratum mycelium but no spore formation evident. Unstained, living material. × 101.

FIG. 21. *Act. thermophilus*. Unstained living material at incipient stage of spore formation. Tips of hyphae showing indentation. × 420.

FIG. 22. *Act. thermophilus*. Unstained living preparation, 10 days old, on Czapek's agar. Note complete spore formation. × 420.

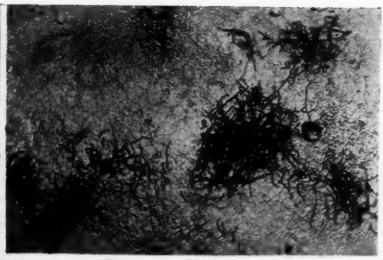
FIG. 23. *Act. thermofuscus*. Stained with methylene blue, showing spirals and formation of spores. × 975.

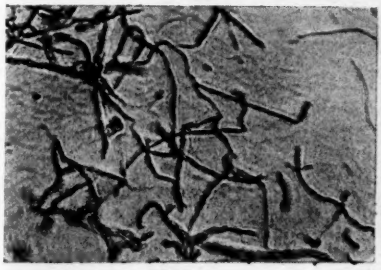
FIG. 24. *Micr. vulgaris*. Gram stain of an 18-hour slide culture (60°C.), showing germinating spores and initial mycelium formation. × 975.

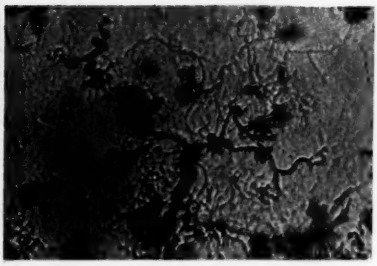
FIG. 25. *Micr. vulgaris*. Early stages of spore formation. Gram stain. Showing swelling of branches and incipient spores. × 975.

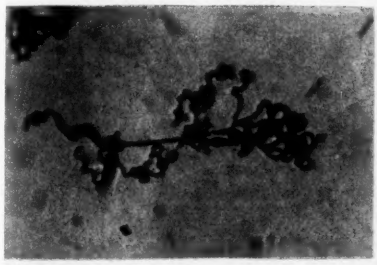
FIG. 26. *Micr. vulgaris*. Later stages of spore formation. Note that spores that have broken away are stained only lightly. × 975.

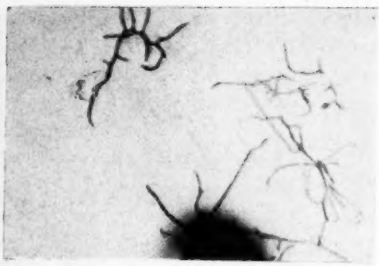
19

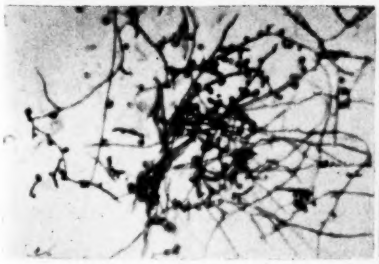

20

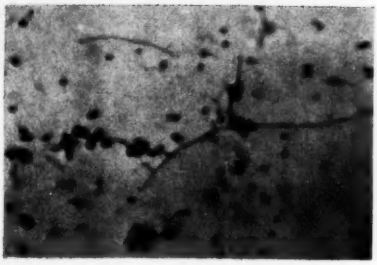

21


22


23


24


25


26

FIGS. 19–26

ROOT NODULE BACTERIA OF SOME TROPICAL LEGUMINOUS PLANTS: II. CROSS-INOCULATION TESTS WITHIN THE COWPEA GROUP[1]

O. N. ALLEN AND ETHEL K. ALLEN

University of Hawaii

Received for publication June 7, 1938

In 1936 the authors (3) reported the addition of numerous tropical leguminous species to the cowpea group on the basis of positive reciprocal cross-inoculation with the cowpea plant (*Vigna sinensis* L.). The results presented in this report concern the cultural and morphological characteristics of the rhizobia from the tropical species, and the conclusions drawn from greenhouse tests in which two strains of rhizobia from each plant species were used as inocula for 20 plants now included as members of the cowpea group.

EXPERIMENTAL

Cultural study

The strains of root nodule bacteria used in this study were isolated from leguminous plants which were found growing either in the gardens and arboreta of the Island of Oahu or along the roadsides throughout the Territory of Hawaii. The methods used in the isolation and purification of the nodule-forming cultures were described in the previous report (3).

All cultures consisted of Gram-negative, short rods, varying from 1.0 to 2.0 μ in length, and were slightly swollen in the bacterioidal stage. Young cells were usually uniformly stained, whereas older cultures showed slight variations in cell shapes, sizes, and the usual irregular staining characteristic of rhizobia. Weak carbol fuchsin (Ziehl formula diluted 1–10 with water) was the best stain for observing cell morphology. As a rule the Gram method (Kopeloff and Beerman modification) was not preferred, since the basic fuchsin stained the cells very faintly. Representative cultures of each species stained by the technic developed by Gray (12) showed a monotrichous type of flagellation.

Fifty-four strains of root nodule-forming bacteria isolated from 28 tropical or subtropical leguminous species, as listed in table 1, were studied on five media consisting of mineral salt agar as a base plus the following plant extracts: Bacto-yeast; Bacto-yeast plus potato; asparagus, as described by Carroll (7), plus potato; kraut juice; and kraut juice plus calcium gluconate. The use of

[1] Published with the approval of the director as Technical Paper No. 115 of the Pineapple Experiment Station, University of Hawaii.

kraut juice as a source of accessory growth factors and its combination with calcium gluconate was recently proposed by Albrecht and McCalla (1, 2). The formula of the yeast extract medium plus the potato extract differed from that proposed by Sarles and Reid (20) in that Bacto-yeast was used in a 0.5 per cent concentration instead of the fresh, starch-free, compressed yeast. Fifteen carbohydrates; namely, arabinose, xylose, rhamnose, dextrose, levulose, galactose, sucrose, lactose, maltose, raffinose, dextrin, salacin, mannitol, glycerol, and dulcitol, were added as sources of carbon in combination with each of the five plant extract media. With the exception of the medium proposed by Albrecht and McCalla (2) the mineral salt base consisted of K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; NaCl, 0.1 gm.; and $CaCO_3$, 3.0 gm. per liter. Five cubic centimeters of a 0.5 per cent alcoholic solution of brom-thymol-blue was added to each liter of the plant extract carbohydrate agar medium.

In general the physiological and cultural characteristics of the rhizobia from the tropical species are in agreement with those previously reported and commonly accepted as normal or typical reactions of "cowpea" bacteria. Growth on the Bacto-yeast extract media and the kraut juice media was scant to moderate and very slow. Many of the organisms showed no growth on the kraut juice calcium gluconate medium irrespective of the carbohydrate source. Asparagus extract mannitol agar proved to be the most suitable for isolation and cultural purposes; however, growth of the majority of the strains was considerably augmented on this medium by the addition of potato extract. On the one occasion when fresh starch-free compressed yeast plus potato extract medium was used, the growth compared in general with that obtained on the asparagus-potato medium. In regard to abundance of growth the plant extract media may be arranged in the following ascending order irrespective of the carbohydrate source: kraut juice, kraut juice plus calcium gluconate, Bacto-yeast, Bacto-yeast plus potato extract, asparagus extract, fresh compressed yeast plus potato extract, and asparagus-potato extract.

The majority of the cultures produced a white or colorless, moist, translucent to opaque type of growth on the agar slants. The cultures isolated from *Samanea saman*, *Andira inermis*, *Albizzia lebbek*, *Piscidia erythrina*, *Parkia africana*, and *Cytisus scoparius* were exceptions in that the growth was abundant, raised, and mucilaginous in almost all instances. In general, the type of growth was a more or less constant characteristic regardless of the carbohydrate and the plant extract.

The cultures produced alkaline reactions on all the media with the exception of *Andira inermis* 20-1 and 20-2, which were slightly acid on dextrose. Reversion of reactions occurred with many cultures on xylose and arabinose, the reactions being at first alkaline, later changing to slightly acid. Such reversions were always much slower on the xylose media.

All cultures were tested in duplicate on asparagine mannitol agar plus 0.15 per cent tyrosine as proposed by Stapp (22), and later used by Almon and Fred (4). Only three strains, *Albizzia lebbek* 39-1, *Piscidia erythrina* 75-1, and

Parkia africana 89-1, produced strongly positive reactions. Buff colored or weakly positive reactions resulted with strains 13-5, 36-1, 108-2, and 11.

Reactions produced by the cultures in Bacto-litmus milk were noted at weekly intervals in a series of replicated experiments. Within 3 weeks, each culture produced moderate alkalinity which usually became pronounced as time progressed. Hydrogen-ion concentrations of the milk cultures, as determined by the electrometric method at the end of 1-, 2-, and 3-month periods, showed the final reactions produced by all the strains to vary between pH 8.03 and 8.23. The cultures isolated from *Cassia mimosoides*, *Andira inermis*, *Canavalia campylocarpa*, and *Inga laurina* consistently produced clear, distinct serum zones within the first 6-week incubation period. During the second and third months several others produced a cloudy serum or surface zone of an opacity less than that of the milk. These latter reactions, however, were believed to be due to the continuance of high alkalinity for a long time, and since the watery layers retained a cloudy opaque appearance, they were not regarded as true serum zones. The remainder of the cultures did not show any indication of serum zone formation during the prolonged incubation period.

Cross-inoculation tests

In order to understand better the infective and effective abilities of the rhizobia cultures and to validate their host plants as members of the cowpea group, two strains of rhizobia isolated from each of the leguminous species were used as inocula in greenhouse tests. Twenty leguminous species representing fifteen genera were used as the test plants. Twelve of these species have long been accepted as *bona fide* members of the cowpea group. Four of the remaining species, *Acacia koa*, *Erythrina indica*, *Samanea saman*, and *Stylosanthes guianensis*, were placed in the cowpea group by the authors (3). *Clitoria ternatea*, *Lespedeza stipulacea*, and *Crotalaria spectabilis* were added in 1934 by Carroll (7). For a number of years, *Cytisus scoparius* has been included in the cowpea group with doubt (11). The technic employed in the treatment of the leguminous seed, the greenhouse facilities, and the methods of plant culture used in similar cross-inoculation tests were described in considerable detail in the previous report (3).

Within recent years, data (17, 18, 23) have been presented to show that strains of nodule-forming bacteria may differ in their ability to infect plants grown under different seasonal environments and under different amounts of solar radiation. As the data presented in this report have accumulated over a period of several years, it is perhaps fitting to review the equable meteorological conditions under which these experiments were conducted. Although Honolulu is at sea level in about 21° north latitude and is included within the Torrid Zone, the mean annual temperature is about 76°F. The absence of seasonal variation is evidenced by the fact that the warmest month averages 78°F., and the coldest about 71°. The highest temperature recorded in 41 years at the Honolulu station of the U. S. Weather Bureau was 88°F. and the lowest 55°.

The average daily range is a little over 10°F. This relatively low variation in temperature is due, in part, to the following facts: first, the noon sun is never more than 45° from the zenith; second, the longest day is only 3 hours longer than the shortest day; and third, the vast extent of ocean in all directions modulates the variations to a large extent. There are, on the average, 118 clear, 178 partly cloudy, and 69 cloudy days a year. For the last 26 years there has been an average of 62 per cent of the total hours of sunshine, based on the number possible between sunrise and sunset. The lowest amount, about 58 per cent, is usually recorded in December; and the highest amount, about 69 per cent, usually occurs in August.

The results of the greenhouse tests have been based upon a series of three replicated experiments, in each of which the strains of nodule-forming bacteria were run in triplicate against the various host plants. One set of the experiments consisted of only one leguminous species in combination at one time with each of the various cultures. In the other two plant set-ups two or three species of plants of similar growth habits were grown together in the same jars.

The growing period of the plants varied from 7 to 10 weeks. The fast-growing plants usually showed maximum differences as a result of inoculation in about the sixth week, and they were harvested soon thereafter. A longer period was necessary for such differences to become pronounced with those species exhibiting slower growth due to delayed germination or to the presence of large amounts of reserve food materials in the large thick-coated seed. Inasmuch as seasonal variations were practically nil throughout the experiments it is not deemed necessary to specify the plants comprising each experiment or the combinations used in their composite culturing. In general, those species cultured during the January-April cycle of one year were tested during the May-July period of the next, and during the August-November period in the third test. Since the results of the three tests were in agreement over these periods of the year any irregularities noted in the nodule-producing abilities of the strains cannot be attributed to seasonal variations and their effects upon the physiological status of the plant. It is quite probable, however, that variations with these cultures might occur where the growth of the plants is affected by climatic changes of a greater magnitude. For the sake of brevity, dry weights and nitrogen analyses of the plants have been omitted.

All root systems were examined in trays of water in order to facilitate detection of the small ineffective types of nodules. Contamination in the course of the experiments was rare. In the few instances when such did occur, data on that particular series were discarded.

DATA AND COMMENT

A study of the data expressed in table 1 offers two methods of analysis. First, an index of the relative infective ability of each strain of rhizobia upon the 20 species of test plants, i.e., the extent of strain variation, may be obtained by following the course of the reactions horizontally. Accordingly,

conclusions may be drawn regarding the ability of each strain to enter the tissues of the different plants, and its relative capacity to benefit plant growth. Thus, an evaluation may be placed upon the general suitability of a strain as an inoculant for a number of species. Second, by a study of the data vertically, variations in response of each test plant to the 54 sources of inocula may be ascertained. Such a comparative evaluation of the test plants is advantageous in aiding our understanding of the susceptibilities of a species to nodule-forming bacteria when inocula from different sources are used. Thus, from a summary of the data, conclusions should be apparent regarding the relative infective and effective capacities of each strain of nodule-forming bacteria used as well as the response of each test plant to inoculation.

Strain variation

Considerable variation was noted in the infectiveness and effectiveness of each strain of rhizobia. It is likely that a greater variation of such differences occurs among the organisms of the cowpea group than in the more confined or selective cross-inoculation groups, such as the alfalfa, clover, and pea, since the cowpea group now consists of such a cosmopolitan collection of leguminous species more or less distantly related to one another. Only 13 of the 54 strains of rhizobia produced nodules on all of the plants tested. These included both strains isolated from *Stylosanthes guianensis*, *Enterolobium cyclocarpum*, *Derris microphylla*, *Tephrosia candida*, *T. purpurea*, and one culture isolated from each of *Phaseolus lunatus*, *Cassia mimosoides*, and *Piscidia erythrina*. Seventeen other strains failed to produce nodules on only one test plant. With the exception of *Acacia koa* 15-3 and 15-4 and *Inga laurina* 108-1, this failure to produce nodules occurred on the test plant *Phaseolus lunatus*. The strains isolated from *Andira inermis* showed the most restricted range of infectiveness, inasmuch as each failed to nodulate 6 of the 20 species.

Such variations in the infectiveness of root nodule bacteria from plants of a single cross-inoculation group have been noted by other investigators. Carroll (7) in 1934 noted irregularities in the infectiveness of rhizobia from *bona fide* plant members of the cowpea group. His data show that in one instance a velvet bean culture (isolated from *Stizolobium deeringianum*) produced nodules on six plant species included within the cowpea group yet did not nodulate three other plant members. In another instance a strain isolated from cowpea produced nodules on cowpea and peanut but did not produce nodules on beggarweed, hyacinth bean, jack bean, Lespedeza, and lima bean. Conklin (9) obtained similar results. Her data show that of 76 strains of rhizobia isolated from species of eight genera included in the cowpea group, 58 strains were infective and 12 were noninfective on the cowpea plant. Erratic or doubtful results in the nodulation of the cowpea plant were also obtained with six strains isolated from *Baptisia tinctoria* and *Cassia nictitans*. Raju (16) noted variations in the ability of rhizobia from members of the cowpea group to nodulate species cultured under the best growing conditions. His data show that

TABLE 1

Results of inoculation of plant members of the cowpea group by rhizobia from tropical leguminous species

HOST ISOLATIONS	Strain number	TEST PLANTS: *Acacia koa*	*Cajanus cajan*	*Canavalia ensiformis*	*Clitoria ternatea*	*Crotalaria juncea*	*Crotalaria speciabilis*	*Cytisus scoparius*	*Desmodium purpureum*	*Dolichos lablab*	*Erythrina indica*	*Lespedeza sericea*	*Lespedeza stipulacea* (Korean)	*Lespedeza stipulacea* (Harbin)	*Phaseolus aureus*	*Phaseolus aconitifolius*	*Phaseolus lunatus*	*Samanea saman*	*Stizolobium utile*	*Stylosanthes guianensis*	*Vigna sinensis*
Phaseolus lathyroides	1-4	+	++	++	I	+	+	+	I	I	+	I	+	I	+	+	−	+	+	+	++
	1-5	+	+	++	+	+	+	+	I	I	+	I	+	+	+	+	−	+	+	+	++
Phaseolus lunatus	4-3	+	+	+	I	+	+	+	+	I	+	+	+	+	+	+	I	*	I	+	+
Cassia mimosoides	5-2	+	++	++	I	+	+	+	+	+	+	I	+	++	+	+	I	I	+	++	+
	5-3	+	++	++	+	+	+	+	+	+	+	I	I	+	+	+	−	I	+	++	+
Indigofera suffruticosa	7-4	+	++	++	+	+	+	+	+	I	++	−	I	+	I	+	−	++	+	++	++
	7-5	+	+	++	I	+	+	+	+	I	+	−	I	+	+	++	−	++	+	++	++
Samanea saman	13-5	+	++	I	I	+	+	−	+	+	+	+	+	+	+	+	−	++	+	+	+
	13-7	+	++	I	I	+	+	−	+	+	+	++	+	+	+	+	−	+	−	+	+
Acacia koa	15-3	++	I	I	I	+	+	+	+	I	+	+	+	++	+	I	I	I	−	+	++
	15-4	++	I	I	I	+	+	I	+	I	+	+	+	++	+	I	I	I	−	+	+
Andira inermis	20-1	+	I	I	I	I	I	−	−	+	−	+	+	+	−	I	−	I	−	I	+
	20-2	+	I	+	I	I	I	−	−	I	−	I	+	+	−	I	−	I	−	I	+
Desmodium barbatum	22-1	+	+	+	I	++	+	+	+	++	+	+	+	+	+	+	−	++	++	+	+
	22-2	+	+	+	+	++	+	+	+	++	+	++	−	+	++	+	−	+	++	++	+
Stylosanthes guianensis	23-1	++	++	++	++	+	+	+	+	++	++	+	+	++	+	+	I	+	++	++	+
	23-3	++	+	++	+	+	+	+	+	++	+	+	+	+	++	++	I	+	++	++	+
Lespedeza sericea	25-4	++	++	++	I	++	++	+	−	+	++	+	+	+	+	+	−	+	+	+	+

Lespedeza sericea	25-4	++	++	++	I	++	++	+	−	+	++	+	+	+	++	++	−	++	++	+	++
Vigna marina	27-1	I	I	+	I	+	I	−	−	+	+	+	+	+	+	I	−	I	−	++	++
	27-2	I	I	+	I	+	+	−	−	I	+	+	++	+	+	I	I	I	−	++	++
Canavalia campylocarpa	32-6	++	++	+	I	+	+	+	+	+	+	+	+	+	+	+	−	+	+	++	+
	32-7	++	+	+	++	+	+	+	+	+	+	+	+	+	+	+	−	+	+	++	+
Enterolobium cyclocarpum	34-7	++	I	+	I	+	+	+	+	I	+	+	+	+	+	+	I	++	I	I	++
	34-10	++	+	+	+	+	+	+	+	I	+	+	+	+	+	+	I	++	I	I	++
Erythrina indica	36-1	++	++	I	++	++	+	+	+	+	+	+	++	+	+	+	−	I	+	I	++
	36-4	++	++	I	+	++	+	+	+	+	++	+	++	+	+	+	−	I	+	I	++
Albizzia lebbek	39-1	+	+	++	−	+	++	+	−	+	+	+	+	++	+	+	−	I	I	+	++
	39-3	+	+	++	−	+	++	+	−	+	+	+	+	+	+	+	−	I	I	+	++
Lonchocarpus domingensis	74-1	I	++	++	++	+	++	*	+	+	+	*	+	+	+	+	−	I	−	I	+
	74-5	I	+	++	I	+	++	+	+	+	+	+	+	+	+	+	−	I	−	I	++
Piscidia erythrina	75-1	++	++	I	I	I	+	+	+	++	+	+	+	+	+	++	−	+	I	+	++
	75-4	+	++	+	I	I	+	I	+	+	+	+	+	+	+	++	I	+	I	+	++
Alysicarpus vaginalis	77-3	++	+	+	+	+	+	+	+	++	+	+	+	+	+	+	−	I	+	++	++
	77-6	++	+	+	+	+	+	−	+	++	++	+	+	+	+	+	−	I	+	++	++
Derris microphylla	86-2	+	++	++	I	+	+	+	+	I	+	I	I	+	+	+	I	+	+	++	++
	86-3	++	+	++	I	+	+	+	+	+	*	+	I	+	+	+	I	+	+	++	++
Pongamia pinnata	87-2	+	++	++	I	+	+	+	+	++	+	+	I	+	+	+	−	I	I	++	++
	87-4	++	++	++	I	+	+	+	+	+	+	+	+	+	+	+	−	+	I	++	++
Parkia africana	89-1	I	++	+	I	+	+	I	−	++	+	−	+	+	+	+	−	I	+	I	+
	89-2	I	++	+	I	+	+	+	−	++	+	−	+	+	+	+	−	I	+	I	+

TABLE 1—*Concluded*

HOST ISOLATIONS	Strain number	TEST PLANTS: *Acacia koa*	*Cajanus cajan*	*Canavalia ensiformis*	*Clitoria ternatea*	*Crotalaria juncea*	*Crotalaria speciabilis*	*Cytisus scoparius*	*Desmodium purpureum*	*Dolichos lablab*	*Erythrina indica*	*Lespedeza sericea*	*Lespedeza stipulacea* (Korean)	*Lespedeza stipulacea* (Harbin)	*Phaseolus aureus*	*Phaseolus aconitifolius*	*Phaseolus lunatus*	*Samanea saman*	*Sesbania sesban*	*Stylosanthes guianensis*	*Vigna sinensis*
Tephrosia candida	99-1	++	+	++	+	++	+	+	+	I	+	I	I	+	+	+	I	++	+	+	++
	99-3	+	+	++	+	++	+	+	+	I	+	I	I	++	++	+	I	++	+	+	++
Hymenaea coubaril	100-1	++	I	I	I	+	+	+	−	++	+	−	−	I	+	I	I	I	−	+	+
	100-2	++	I	I	I	+	+	I	−	+	+	−	−	I	+	I	I	I	−	+	+
Tephrosia purpurea	107-1	++	++	+	+	++	++	+	+	I	+	+	+	+	+	+	I	+	++	++	++
	107-3	++	++	+	++	++	++	+	+	I	++	+	+	+	+	+	I	+	++	++	++
Inga laurina	108-1	I	++	+	I	+	++	+	−	++	+	+	+	+	+	+	I	I	++	+	++
	108-2	+	++	+	I	+	++	−	−	++	+	+	I	+	+	+	I	I	++	+	++
Cytisus scoparius	110-1	*	I	+	−	++	−	+	+	+	+	+	+	+	+	++	I	*	−	+	+
	110-2	*	I	I	−	+	−	+	+	++	+	+	+	+	+	+	I	*	−	+	+
Tephrosia noctiflora	115-2	++	++	++	+	++	+	I	+	I	++	+	+	+	+	+	−	++	++	++	++
	115-4	++	+	+	++	+	++	+	+	+	+	++	+	+	+	+	−	+	++	++	++
Vigna sinensis	10	++	++	++	+	++	++	+	−	+	++	+	+	+	++	++	−	++	++	+	++
	11	*	+	+	+	+	+	I	−	++	+	+	+	+	+	+	−	+	I	+	+
Summary of test plant reactions																					
No nodules formed	−	0	0	0	4	0	2	8	15	0	2	6	3	0	2	0	32	0	13	0	0
Ineffective responses	I	7	11	11	29	4	3	6	2	17	0	8	9	3	1	8	22	23	10	10	0
Effective responses: Fair	+	20	18	22	15	38	38	39	37	22	43	36	39	45	46	39	0	17	19	23	22
Effective responses: Good	++	24	25	21	6	12	11	0	0	15	8	3	3	6	5	7	0	11	12	21	32

* Insufficient data.

strain 600, isolated from the cowpea (*Vigna sinensis*), produced nodules on eight plant members of the cowpea group but failed to infect four other plant species. On the other hand, a strain isolated from *Phaseolus aconitifolius* was found by Raju to nodulate all the plants tested.

Several occasions of nonreciprocal cross-inoculation are shown in the data of table 1. For example, *Lespedeza sericea* 25-4 failed to produce nodules on *Phaseolus lunatus;* yet strain 4-3 from *Phaseolus lunatus* not only produced nodules on *Lespedeza sericea* but benefited plant growth. Similar reactions were noted in the results obtained with reciprocal inoculations with cultures and plants of *Erythrina indica*, *Vigna sinensis*, and *Phaseolus lunatus*.

Seven instances are noted in table 1 wherein the two strains isolated from the same host plant did not produce nodules on the same test plants. As an example, *Cassia mimosoides* 5-2 was highly infective on the lima bean, as evidenced by the formation of numerous small nodules over the root system, yet strain 5-3 isolated from the same species, although one growing in a different locality, failed to produce nodules on the lima bean. Although it may be conjectured that such inconsistencies are due to technic or environmental variations in greenhouse culture, such an explanation seems improbable, since a plant species was tested against all sources of inocula within each experiment, and such experiments were maintained under as nearly identical conditions as possible. The replication of these results in the experiments in which the species were grown singly and in combination with other species whose roots were nodulated suggests strongly that discrepancies in uniformity of nodulation might be due to the inherent capacity of one particular strain to enter the tissues of the respective plants in question and the lack of such a capacity by the other strain.

Various irregularities of similar nature have also been noted by previous investigators. Burrill and Hansen (5), as early as 1917, noted that a strain of rhizobia from cowpea failed to nodulate partridge pea (*Cassia chamaecrista*), although all the other cowpea strains were definitely infective on this species. Carroll's results (7) show that Lespedeza 5 failed to produce nodules on cowpea and lima bean plants, although Lespedeza 4 was infective on both species. A similar example occurred with the strains isolated from cowpea plants; cowpea 28 produced nodules on the hyacinth bean, but did not infect jack bean; cowpea 10 was infective on both species, yet cowpea 11 was noninfective on both species. Conklin's data (9) show that some of the strains isolated from *Baptisia tinctoria*, *Desmodium paniculatum*, and *Genista tinctoria* did not infect the cowpea plant. Although it is not definitely stated by the aforementioned authors that these nonconformities of inoculation occurred within the optimum growing seasons, such data do serve as evidence that various strains isolated from the same host plant may show definite host specificities in regard to this aspect of infectiveness.

In the summary of strain reactions contained in table 2 it is of interest to note that the majority of rhizobia strains from the various tropical species were

* Insufficient data.

beneficial on the test plants. Forty-eight, or 89 per cent, of the strains enhanced plant growth and stimulated the fixation of appreciable amounts of nitrogen in more than 10 different plant species. Twenty-five of these strains benefited 16 or more of the different plants; four of these, as cited in table 1; namely, *Stylosanthes guianensis* 23-1, 23-3, *Canavalia campylocarpa* 32-7, and *Tephrosia noctiflora* 115-4, were effective upon 19 species. *Andira inermis* 20-1 and 20-2 and *Phaseolus lunatus* 4-3 were the only strains which were not outstandingly effective on a single host plant.

Four strains, *Desmodium barbatum* 22-2, *Canavalia campylocarpa* 32-7, *Tephrosia noctiflora* 115-4, and *Vigna sinensis* 10, did not produce a single case of ineffectiveness. Each of the species infected by these strains showed the presence of large nodules on the taproot system, and the plants exhibited luxuriant growth and dark green foliage. All other strains were nonbeneficial on one or more of the test plants, although only nine strains were noneffective on more than five. The two strains isolated from *Andira inermis* were the

TABLE 2

Summary of strain reactions

NUMBER OF STRAINS	PERCENTAGE OF TOTAL	REACTION ON TEST PLANTS
41	75.9	Noninfective on 1–6 test plants
13	24.1	Infective on all test plants
41	75.9	Nonbeneficial on 1–5 test plants
9	16.6	Nonbeneficial on 6–9 test plants
6	11.1	Beneficial on 1–10 test plants
23	42.6	Beneficial on 11–15 test plants
25*	46.2	Beneficial on 16–19 test plants

* Four strains were beneficial on 19 test plants; none of the strains were infective and effective on all test plants.

most conspicuous of the poor or least desirable sources of inocula. Eight of the fourteen different species nodulated by *Andira inermis* 20-1 showed small, ineffective nodules, yellow foliage, poor growth and no appreciable fixation of nitrogen. It is of interest from the standpoint of host specificity that strain 20-1 was ineffective on *Canavalia ensiformis* and beneficial on *Dolichos lablab* and *Lespedeza sericea;* whereas strain 20-2 was effective on *Canavalia ensiformis* and nonbeneficial on the other two species.

Test plant reactions

A summary of the reactions showing the response of each species to the various inocula appears at the end of table 1. Only 10 of the 20 plant species, namely: *Acacia koa, Cajanus cajan, Canavalia ensiformis, Crotalaria juncea, Dolichos lablab,* the Harbin variety of *Lespedeza stipulacea, Phaseolus aconitifolius, Samanea saman, Stylosanthes guianensis* and *Vigna sinensis* were nodulated by each of the 54 strains of nodule bacteria. In contrast, 32 strains

failed to produce nodules on *Phaseolus lunatus*, 15 failed on *Desmodium purpureum*, and 13 on *Stizolobium utile*. Similar results in regard to the relative susceptibilities of *Vigna sinensis* and *Phaseolus lunatus* to infection by rhizobia have been reported by Carroll (7).

With the exception of three species, *Clitoria ternatea*, *Phaseolus lunatus*, and *Samanea saman*, every test plant was benefited by 31 or more of the different nodule-forming strains. *Vigna sinensis* was the only species upon which every strain formed nodules and enhanced plant growth. Similarly, all of the strains producing nodules upon *Erythrina indica* were beneficial, but two strains, *Andira inermis* 20-1 and 20-2, failed to infect this species. *Phaseolus lunatus* and *Clitoria ternatea* may be considered as examples of the other extreme, inasmuch as each of the 22 strains producing nodules on the former species proved nonbeneficial, and 29 of the 50 strains infecting the latter failed to enhance plant growth or to stimulate the fixation of atmospheric nitrogen.

To date the division of a plant genus between more than one cross-inoculation group is attributed to only three genera, Vicia (7), Lupinus (7), and Phaseolus (7, 11). In regard to the last, eight species are included in the cowpea group, and three other species constitute the bean group. The results of this study confirm the addition of *Phaseolus lathyroides* to the cowpea group, since each of the strains from this species produced nodules on 19 of the 20 test plants. It is of interest to note that *Phaseolus lunatus*, a related species, was the only test plant not nodulated by each of these strains.

Whether or not the species of the genus Tephrosia are to be divided between two cross-inoculation groups is yet to be determined. Recently Bushnell and Sarles (6) reported that the cultures isolated from *Tephrosia virginiana* var. *holosericea*, found in Wisconsin, did not form nodules upon the cowpea plant or any other plants tested. Accordingly, this species was placed by them in the list of plants unassigned to any cross-inoculation group. On the other hand the authors (3) have reported that reciprocal cross-inoculation occurs between the cowpea and three tropical species, *T. candida*, *T. purpurea*, and *T. noctiflora*. The data in table 1 show that the two strains isolated from each of these species also exhibit a rather marked affinity for the plant members of the cowpea group, since, with the exception of *T. noctiflora* 115-2 and 115-4, the Tephrosia cultures produced nodules on each of the 20 test plants. Recently Raju (16) reported that *T. purpurea* was nodulated by rhizobia from the cowpea and *Phaseolus aconitifolius*.

In several instances certain strains of rhizobia, when used as inocula on *Phaseolus aconitifolius*, *Samanea saman*, and *Stizolobium utile*, showed a "latent or delayed state of effectiveness." This was usually a condition wherein the plants underwent a prolonged period of nitrogen hunger, which was followed somewhat suddenly, that is, within several days, by rapid growth accompanied by changes in the color of the leaves from yellow to dark green. These changes in vigor and color of the plants were taken as an indication of an immediate availability of nitrogen from the nodules. The replication of such reactions in experiments conducted under cellophane covers showed that this latent

response was not due to nodule formation resulting from contamination. It is likely that an arrested equilibrium between the plant and the availability of nitrogen in the nodules may be the explanation.

A perspective of infective variations of rhizobia

Within recent years considerable data have accumulated concerning variations in the infective abilities of rhizobia. The occurrence of irregularities in the nodule-producing capacity of rhizobia isolated from the *same* species, as heretofore mentioned in this report, presents a situation somewhat analogous to those previously reported regarding the interchangeability of nodule-forming bacteria from cowpea, soybean, and lupine plants. Hansen and Tanner (14), Leonard (15), and Sears and Carroll (21) have shown that many strains of soybean bacteria produce nodules on the cowpea plant, whereas fewer cowpea cultures tested to date have been able to produce nodules on the soybean. In some instances (8) soybean bacteria have been as effective upon cowpea plants as were efficient cowpea strains, although not so commonly has the reverse been true. The contributions of Reid (18) and of Bushnell and Sarles (6) are especially enlightening in regard to the relationship between the cowpea, soybean, and lupine cross-inoculation groups.

It appears logical from a purely evolutionary point of view that in some instances strains of rhizobia may have developed rather specialized adaptations or affinities for particular host plants, whereas others have not. Within those cross-inoculation groups consisting of a few closely related species, it is probable that this specialization has reached a more unified status, and therefore such strains have lost, or probably never had, affinities for plants outside of a few genera. Yet it is likely that as additional plants are investigated those cross-inoculation groups now confined to a few plant species will be enlarged. Specialization of rhizobia in regard to host plants has probably not yet occurred with many of the strains from species bearing a relationship to the cowpea, inasmuch as so many distantly related leguminous plants are apparently concerned.

Such assumptions concerning specialization, adaptation of rhizobia to host plants, and similar conditions become theory, since facts regarding the origin of nodule-forming bacteria and their relationships with certain species are not well understood. Consequently, a question arises as to whether a plant species can be considered the "true parent host" of the nodule-forming bacteria which are present in the nodules of its root system. A similar question arises as to whether it is to be implied that all strains isolated from a particular host plant do or should demonstrate affinities for the same plant species. Within the alfalfa, clover, and pea groups no occasion has been reported wherein the rhizobia have not been mutually interchangeable on the plant members of the respective groups; however, the ability of strains to nodulate certain plants and not others has been shown to exist among the nodule-forming bacteria isolated from various plant members of the cowpea group. Whether specific strains of nodule-forming bacteria from a particular plant species should be

considered representative of all the rhizobia from that species is problematical. Especially within the cowpea group does versatility in the infective ability of a strain become a factor of consideration, since the data heretofore presented illustrate the wide range of susceptibility of the cowpea plant to nodulation. At the present time more than 125 leguminous plants have been shown to be the hosts of rhizobia capable of producing nodules on the cowpea plant.

Although several investigators (13, 19) have claimed that more than one strain of rhizobia may be isolated from the same nodule, more recent data (10) have not been confirmatory. Yet it is agreed that different strains of rhizobia may produce nodules on a leguminous plant at approximately the same time. In accepting this latter condition, one must assume that the isolation of nodule-forming bacteria from one nodule does not afford a true representation of all the different strains which may be present in other nodules on the same species. Especially in regard to the plants of the cowpea group does it seem logical to assume that some of the strains infecting a plant growing under natural conditions in field or forest soil may be mutually interchangeable with certain species, whereas other strains infecting the same host may bear little or no affinity for these species, yet these may be mutually interchangeable on another collection of closely related species. What effect, if any, the chemical and physical environment of a plant *per se* has upon the ability of a strain of rhizobia to infect other species has not been shown on an experimental basis. The conception of a cross-inoculation group as "a collection of plants among which the nodule-forming bacteria are mutually interchangeable" appears fundamental from a classification and practical standpoint. Yet, this interpretation should not be considered so biologically true that the ability of a strain to nodulate some host plants and not others within a group may be regarded as inconsequential.

SUMMARY

A study has been made of the morphological and physiological characteristics of 54 strains of root nodule-forming bacteria isolated from 28 different leguminous plants growing in Hawaii. Reactions of these strains were noted in litmus milk and on five plant extract media in combination with fifteen carbohydrates.

Cross-inoculation tests using these strains as sources of inocula for 20 plant members of the cowpea group have shown marked variations in the infectiveness and effectiveness of the rhizobia. Only about 25 per cent of the strains produced nodules on all of the test plants. In regard to benefits derived from the inoculation, 48, or 89 per cent, of the strains were beneficial on more than 10 plant species, 4 of the strains being beneficial on 19 species.

Ten of the test plants were nodulated by all sources of inocula. *Vigna sinensis* was the most susceptible, inasmuch as each strain of rhizobia not only produced nodules but enhanced the growth of the plant. The other extreme was represented by *Phaseolus lunatus* on which only 22 strains produced nodules, none of which were beneficial.

The results suggest that variations in the infectiveness and effectiveness of

rhizobia from plants of the cowpea group may be of a greater magnitude than those previously recorded for the other cross-inoculation groups.

REFERENCES

(1) ALBRECHT, W. A., AND MCCALLA, T. M. 1937 Adsorbed calcium on colloidal clay and an accessory growth factor in laboratory production of Rhizobium cultures. *Jour. Bact.* 33: 80–81 (abs.).

(2) ALBRECHT, W. A., AND MCCALLA, T. M. 1937 A new culture medium for rhizobia. *Jour. Bact.* 34: 455–457.

(3) ALLEN, O. N., AND ALLEN, E. K. 1936 Root nodule bacteria of some tropical leguminous plants: I. Cross-inoculation studies with *Vigna sinensis* L. *Soil Sci.* 42: 61–77.

(4) ALMON, L., AND FRED, E. B. 1933 The production of tyrosinase among various species of rhizobia and related organisms. *Centbl. Bakt.* (II) 88: 302–304.

(5) BURRILL, T. J., AND HANSEN, R. 1917 Is symbiosis possible between legume bacteria and non-legume plants? *Ill. Agr. Exp. Sta. Bul.* 202: 115–181.

(6) BUSHNELL, O. W., AND SARLES, W. B. 1937 Studies on the root-nodule bacteria of wild leguminous plants in Wisconsin. *Soil Sci.* 44: 409–423.

(7) CARROLL, W. R. 1934 A study of Rhizobium species in relation to nodule formation on the roots of Florida legumes: I. *Soil Sci.* 37: 117–135.

(8) CARROLL, W. R. 1934 A study of Rhizobium species in relation to nodule formation on the roots of Florida legumes: II. *Soil Sci.* 37: 227–241.

(9) CONKLIN, M. E. 1936 Studies on the root nodule organisms of certain wild legumes. *Soil Sci.* 41: 167–185.

(10) DUNHAM, D. H., AND BALDWIN, I. L. 1931 Double infection of leguminous plants with good and poor strains of rhizobia. *Soil Sci.* 32: 235–249.

(11) FRED, E. B., BALDWIN, I. L., AND MCCOY, E. 1932 Root nodule bacteria and leguminous plants. Univ. Wis. Studies Sci. 5.

(12) GRAY, P. H. H. 1926 A method of staining bacteria flagella. *Jour. Bact.* 12: 273–274.

(13) GREIG-SMITH, R. 1906 The formation of slime or gum by *Rhizobium leguminosarum*. *Linn. Soc. N. S. Wales, Proc.* 31: 264–294.

(14) HANSEN, R., AND TANNER, F. W. 1931 The nodule bacteria of the Leguminosae with special reference to the mechanism of inoculation. *Centbl. Bakt.* (II) 85: 129–152.

(15) LEONARD, L. T. 1923 Nodule-production kinship between the soybean and the cowpea. *Soil Sci.* 15: 277–283.

(16) RAJU, M. S. 1936 Studies on the bacterial-plant groups of cowpea, Cicer, and Dhaincha. I. Classification. *Centbl. Bakt.* (II) 94: 249–262.

(17) RAJU, M. S. 1936 Studies on the bacterial-plant groups. II. Variations in the infective power of the nodule bacteria of cowpea group. *Centbl. Bakt.* (II) 94: 337–348.

(18) REID, J. J. 1936 The infective ability of rhizobia of the soybean, cowpea and lupine cross-inoculation groups. Ph.D. Thesis, Univ. Wis.

(19) ROSSI, G. DE 1907 Ueber die Microorganismen welche die Würzelknöllchen der Leguminosen erzeugen. *Centbl. Bakt.* (II) 18: 289–314; 418–488.

(20) SARLES, W. B., AND REID, J. J. 1935 Growth stimulants for certain rhizobia. *Jour. Bact.* 30: 651 (abs.).

(21) SEARS, O. H., AND CARROLL, W. R. 1927 Cross-inoculation with cowpea and soybean nodule bacteria. *Soil Sci.* 24: 413–419.

(22) STAPP, C. 1923 Beiträge zum Studium der Bakterientyrosinase. *Biochem. Ztschr.* 141: 42–69.

(23) WILSON, P. W. 1935 The carbohydrate-nitrogen relation in symbiotic nitrogen fixation. Wis. Agr. Exp. Sta. Res. Bul. 129.

PHYSICAL CHARACTERISTICS OF SOILS: II. EXPRESSING MECHANICAL ANALYSIS AND STATE OF AGGREGATION OF SOILS BY SINGLE VALUES

AMAR NATH PURI AND B. R. PURI

Irrigation Research Institute, Lahore, India

Received for publication August 17, 1937

Size distribution curves of soils afford the most comprehensive picture of the mechanical composition of such aggregates. Such curves, though characteristic of different soils, are not convenient for defining soils for purposes of classification. An attempt was made to express the mechanical composition of soils by "single values" derivable from the summation or distribution curves. There are three such values, each characteristic of the shape of the distribution curve, as follows:

Weighted mean size, M.—The summation curve of a soil sample gives the percentage of particles below any given size (fig. 1). By taking the readings for two given sizes and subtracting, we can ascertain the percentage of particles whose diameters lie between the two sizes and which may very nearly be assumed as having a diameter lying midway between those two sizes. If this last value is multiplied by the corresponding percentage, and the sum of all such products is divided by the sum of percentages (usually 100), we get a value for the mean diameter of all the particles contained in the sample. This is the weighted mean size, or M, and furnishes a useful measure of the degree of coarseness of the sample.

Standard deviation, σ.—Not every sample consists of particles distributed in exactly the same way, and it is quite possible that two samples with the same mean size may differ, one having a preponderance of particles with diameters near the mean size, and the other with diameters varying much more widely. Hence it is necessary to know how the various sizes are distributed about the mean size, and consequently the standard deviation is calculated as a measure of their dispersion. To obtain this, the deviation of each size from the weighted mean size is squared and multiplied by the corresponding percentage and then the sum of such products is divided by the sum of the percentages. The square root of the quotient gives σ, and the smaller it is the more uniform can the sample be assumed to be.

Schoklitsch number, or K.—The maximum diameter of soil particles for an ordinary sample may be taken as 1 mm. In practice, therefore, the summation curve is bounded by the 0 and 1-mm. ordinates; and the line $R\ P\ S$ may be taken to represent a normal summation curve (fig. 1). If then the area A lying above and to the left of the summation curve is divided by the area B lying below and to the right of the summation curve, the fraction A/B remains constant as long as the limit 0 and 1.0 remains unaltered. This fact was pointed out by Professor Schoklitsch. The constant is referred to as the Schoklitsch number, or K, and when not otherwise specified is taken to refer to diameters lying between 0 and 1.0 mm. Should it be desired to specify these limits more particularly it may be written 0^{k} 1.0; in the same way 0^{k} 2.0 would refer to mixtures whose summation curves lie between 0 and 2.0 mm.

In dealing with the size distribution of particles in soils we must take note of the fact that the state of aggregation as it is found in nature may be very different from its ultimate structure. The former denotes tilth, and the latter

provides a datum line for expressing the limits of variations in soil texture. It must be remembered that the crumb structure of the soil is merely a single phase in its dynamic history, and therefore it cannot be used for the textural classification of soils which must be based on their ultimate structure.

Tilth is a complicated property of the soil, the determination of which would be extremely useful from the practical standpoint. An attempt to correlate soil tilth with the dispersion coefficient was made in a previous publication.[1] The dispersion coefficient (*D. C.*) is measured by determining the percentage of conventional clay (0.002 mm. diameter) by the pipette method after leaving the soil in contact with water for 24 hours (referred to as the dispersion factor, or *D. F.*) and expressing it as percentage of the total clay content of the soil obtainable on complete dispersion. In other words, $D.C. = \frac{D.F. \times 100}{\text{clay content}}$. The dispersion coefficient, therefore measures the percentage of total clay that can pass into the suspensoid state by simple contact with water; and its value

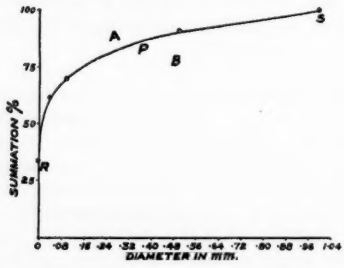


FIG. 1. EXAMPLE OF A SUMMATION CURVE

varies from 0 to 100 per cent, depending on whether the soil is completely flocculated or completely dispersed.

The usefulness of the dispersion coefficient for expressing soil tilth is based on the fact that crumb structure is usually formed by the cementing action of the clay particles, and if these exist in the individual state, all other particles may be present as independent units. In extreme cases, however, it is likely to lead to absurd results. When a small percentage of clay is present in a proportionately large amount of coarser particles the soil as a whole may be in good tilth even when the dispersion coefficient is 100 per cent. The most logical method of expressing soil tilth would be one based on the entire mechanical analysis of the soil or a function thereof. In other words, tilth or the state of aggregation of a soil must give the existing mechanical analysis of the soil as a function of its ultimate analysis. For this purpose M, K, and σ, which are characteristic constants of any mechanical composition curve, could be used. We can take these values of a soil before dispersion and after dis-

[1] Puri, A. N. 1930 Studies in soil colloids: II. Factors influencing the dispersion of soil colloids in water. *Mem. Dept. Agr. India, Chem. Ser.* 2 (2): 39.

persion, the latter values referring to the ultimate structure. The ratio of these constants might be related to soil tilth. We could then see which of these ratios is the most suitable from the practical standpoint.

EXPERIMENTAL

Air-dry soils passing a 1-mm. mesh were used for this study. The mechanical analysis before dispersion (referred to as the aggregate analysis) and after

TABLE 1

Single-value constants relating to the mechanical analyses of soils

SOIL NUMBER	CLAY, PER CENT (0.002 MM.)		DISPERSION COEFFICIENT	MEAN DIAMETER		RATIO M_0/M	STANDARD DEVIATION		RATIO σ_0/σ	SCHOKLITSCH NUMBER		RATIO K_0/K
	Before dispersion	After dispersion		Before dispersion M_0	After dispersion M		Before dispersion σ_0	After dispersion σ		Before dispersion K_0	After dispersion K	
P.C. 1	3.43	11.48	29.88	0.0452	0.0284	1.59	0.0556	0.0450	1.24	0.0473	0.0292	1.62
P.C. 2	1.50	64.06	2.34	0.2464	0.0361	6.83	0.3044	0.0948	3.21	0.3270	0.0375	8.72
P.C. 3	3.10	68.21	4.54	0.2816	0.0227	12.39	0.3188	0.1020	3.12	0.3920	0.0232	16.89
P.C. 4	4.60	15.56	29.56	0.0581	0.0372	1.56	0.0620	0.0573	1.08	0.0617	0.0380	1.62
P.C. 5	5.73	12.79	44.80	0.0553	0.0376	1.47	0.0550	0.0452	1.21	0.0585	0.0390	1.50
P.C. 6	3.68	29.73	12.38	0.1464	0.0432	3.39	0.1820	0.0840	2.17	0.1720	0.0451	3.81
P.C. 7	21.03	22.80	92.24	0.0512	0.0338	1.51	0.1160	0.0434	2.67	0.0540	0.0350	1.54
P.C. 8	2.00	27.30	7.33	0.2784	0.2784	1.00	0.2712	0.3056	0.89	0.3860	0.3860	1.00
P.C. 9	1.88	22.90	8.21	0.2976	0.2396	1.24	0.2876	0.2880	1.00	0.4240	0.3151	1.35
P.C. 10	1.08	38.62	2.80	0.2268	0.1400	1.62	0.2568	0.2256	1.12	0.2930	0.1628	1.80
P.C. 11	0.30	35.08	0.86	0.2172	0.1852	1.17	0.2200	0.2516	0.87	0.2870	0.2273	1.26
P.C. 12	1.13	4.18	27.03	0.1380	0.0760	1.82	0.1268	0.0896	1.42	0.1600	0.0823	1.94
P.C. 13	3.05	66.13	4.61	0.3836	0.0380	10.09	0.4332	0.1060	4.09	0.6220	0.0395	15.77
P.C. 14	1.08	26.96	4.01	0.2976	0.0936	3.18	0.2616	0.1640	1.59	0.4240	0.1033	4.11
P.C. 15	1.00	23.51	4.25	0.2632	0.1080	2.44	0.2080	0.1912	1.09	0.3570	0.1212	2.95
P.C. 16	2.98	7.63	39.06	0.1672	0.1164	1.44	0.1144	0.1104	1.04	0.2010	0.1317	1.53
P.C. 17	2.00	14.46	13.84	0.0924	0.0408	2.26	0.1064	0.0488	2.18	0.1020	0.0425	2.40
P.C. 20	0.90	7.29	12.37	0.1520	0.1372	1.11	0.1212	0.1244	0.97	0.1790	0.1590	1.13
P.C. 25	0.80	4.03	19.86	0.1316	0.1340	0.98	0.0784	0.0724	1.08	0.1520	0.1550	0.98
P.C. 26	0.60	30.97	1.97	0.2952	0.2308	1.28	0.2144	0.2248	0.95	0.4190	0.300	1.40
P.C. 33	1.36	3.70	36.76	0.3280	0.3328	0.99	0.2632	0.2580	1.02	0.4880	0.5000	0.98
P.C. 43	2.00	19.71	10.15	0.0506	0.0280	1.81	0.0564	0.0452	1.25	0.0535	0.0290	1.84
P.C. 44	1.20	8.39	14.30	0.0640	0.0488	1.31	0.0580	0.0388	1.49	0.0680	0.0510	1.33
P.C. 45	1.50	10.68	14.04	0.0616	0.0384	1.60	0.0736	0.0520	1.42	0.0656	0.0400	1.64
P.C. 48	2.56	19.79	12.94	0.1192	0.0396	3.01	0.1224	0.0448	2.73	0.1350	0.0410	3.29

dispersion (ultimate analysis) was made partly by the pipette method (particles below 0.06 mm.) and partly in the Puri siltometer.[2] This siltometer is based on the principle of grading particles by allowing them to fall through a long column of water held in a brass tube 2 m. long, with an internal diameter of 2.5 inches, and collecting the different fractions in separate boxes that move

[2] Puri, A. N. 1935 A siltometer for studying the size distribution of silts and sands. *Punjab Irrig. Res. Inst. Res. Pub.* 2 (7).

into position under water at predetermined intervals of time. The siltometer can deal with particles up to 0.6 mm. diameter; larger particles were graded by sieving.

Mean diameters, standard deviations, and Schoklitsch numbers were calculated for the two sets of values, M_0, σ_0, and K_0 referring to the aggregate analysis, and M, σ, and K to the ultimate analysis. These values and their ratios are given in table 1.

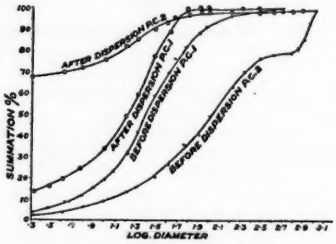


FIG. 2. SUMMATION CURVES OF AN ALLUVIAL SOIL (P.C. 1) AND OF A CHERNOZEM SOIL (P.C. 2)

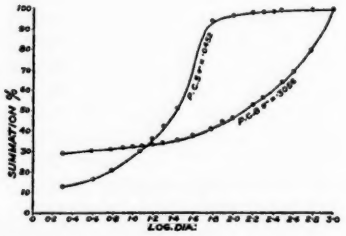


FIG. 3. SUMMATION CURVES OF SOILS TO ILLUSTRATE A LOW AND A HIGH VALUE OF σ

A close examination of these constants with reference to the type of soil reveals the following facts:

Alluvial soils give a low value of M (<0.05 mm.), a low value of σ, and a low value of M_0/M. A low value of M associated with low clay content (below 20 per cent) characterizes an alluvial soil. This is exactly what one would expect. Such deposits are well-graded (low σ), and silt gradually merges into clay. Their low clay content and high silt content do not lead to the formation of many aggregates, a fact that is borne out by a low M_0/M ratio.

Black cotton soils (chernozems) have a high clay content, a low value for M, and a high M_0/M ratio. These soils present a high state of aggregation and possess a crumb structure that would be associated with good tilth.

Lateritic soils give a high M value and a medium M_0/M ratio.

Red ferruginous soils have medium clay, a high M value, a low M_0/M ratio, and a high value for σ. These soils, in fact, behave like a mixture of fine particles abruptly changing into coarse grains.

K values run almost parallel to M values and therefore are not likely to prove any better than the M values.

In order to illustrate the aggregate and ultimate mechanical analysis curves of two types of soils, the values for an alluvial and a black cotton soil P.C. 1 and P.C. 2 are plotted in figure 2. Two typical summation curves that give widely different values of σ are given in figure 3. Note the symmetry of the curve with low σ around the mean diameter as compared to the one with a high σ value.

It is believed that the textural classification of soils on the basis of M and σ will bring out their characteristic differences much more satisfactorily than the usual practice of giving them descriptive names like sandy, loamy, silty, or clayey soil.

The ratio of M_0/M not only brings out the state of aggregation or the crumb structure of a soil but it also shows that the magnitude of this ratio is a characteristic of the soil type. Although theoretically a soil could be made to have almost any value of M_0/M by suitable treatment in the laboratory, natural soils do acquire a stable structure, which, within limits, resists ordinary methods of cultivation. In other words, certain soil types maintain a higher state of aggregation than others. Methods of cultivation of the latter types must be carefully watched, as such soil types are likely to suffer from bad tilth if not handled properly. A word about the relation between dispersion coefficient ($D.C.$) and M_0/M ratio: As may be expected, a high $D.C.$ value goes with a low M_0/M ratio, but since the former refers to only one fraction (clay) the relation is merely qualitative.

SUMMARY

Single-value constants that can be derived from the mechanical analysis summation curves of soils can be used for the textural classification of soils. These values should replace the descriptive terminology generally employed for characterizing soil texture. State of aggregation can be satisfactorily expressed by the ratio of these single values before and after dispersion of the soil.

INFLUENCE OF TEMPERATURE UPON THE MICROBIOLOGICAL POPULATION AND DECOMPOSITION PROCESSES IN COMPOSTS OF STABLE MANURE[1]

SELMAN A. WAKSMAN, T. C. CORDON AND N. HULPOI

New Jersey Agricultural Experiment Station

Received for publication June 30, 1938

In the composting of stable manures and in the preparation of artificial manures from plant residues, the rapidity of evolution of heat and the resulting rise in temperature are tantamount to rapidity of decomposition. It has now been definitely established that microorganisms are largely, if not entirely, responsible for these processes, which finally lead to the transformation of the manure and of the plant materials into a uniform, dark colored mass, generally known as humus. By modifying the temperature of the compost, one is able to control not only the rapidity of the decomposition processes, but also the conservation of the nutrient elements, especially the nitrogen, as well as the nature and effectiveness of the resulting compost. Some of the methods in common use for conserving the nutrients in stable manures are based upon the control of the temperature changes; this is brought about largely through the control of the composition and aeration of the compost.

HISTORICAL

No attempt is made here to review the very extensive literature on the microbiology of stable manures and on the decomposition processes. Attention is directed to only a few contributions which have a direct bearing upon the problem under consideration.

Hébert (5) reported in 1893 that, as a result of decomposition of straw for 3 months at 55°C., there was a loss of one half of the total material. The reduction in cellulose was greater than that of the straw as a whole, whereas that of the vasculose, now known as lignin, was less than that of the straw; a part of the lignin loss was ascribed to its dissolution in the alkaline liquid. Hébert emphasized the fact that the losses of nitrogen as ammonia from manure are apt to be very large, especially under aerobic conditions, and that, under conditions favorable to decomposition, the losses are reduced to a minimum, whereby the ammonia nitrogen is transformed into organic nitrogen. More recently, Russell and Richards (10) emphasized the fact that in the aerobic decomposition of manure in composts, ammonia disappears rapidly; in the presence of sufficient straw, the ammonia is transformed into protein nitrogen, largely through the agency of fungi, without any loss of nitrogen. The rapid losses of nitrogen due to the evaporation of ammonia from the liquid portion of the manure have been emphasized further in a number of recent investigations (12).

As to the specific effects of different temperatures upon the decomposition of organic

[1] Journal Series paper, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

matter, it is sufficient to cite the early work of Dehérain and Demoussy (3). These investigators found that, under optimum moisture conditions, the action of microorganisms upon organic matter increased with an increase in temperature, reaching a maximum at 65°C. Full aeration was shown to be essential for optimum oxidation; an excess of water reduced oxidation considerably.

The microbiological population of stable manures and of composts was found to be very variable in nature, changing continuously, both in numbers and in the specific types of microorganisms, with the progress of the decomposition process. The numerous contributions to this subject do not present, however, a clear picture of the population changes (9). This is true in spite of the fact that numerous bacteria and other microorganisms have been isolated from the composts, ranging from the *Mesentericus ruber* and *Thermophyllis grignoni* of Dehérain (2, p. 783) to *Bact. parvulum* of Conn (1). The action of these pure cultures of bacteria, most of which possess a specific physiology, upon the manure could give only a one-sided picture of the decomposition processes which would normally take place in the manure. Particularly disturbing are the countless enumerations of bacteria found in the manure by many investigators. These numbers, in many instances, had very little bearing upon the processes involved. This resulted in information which was not only far from complete but frequently highly confusing. This information did not seem to point to any correlation between the changing microbial population of the manure and the chemical transformations involved.

The principal reason for the above confusion is to be looked for in the fact that the great majority of investigations dealing with the microbiology of manures and composts were devoted to the study of the bacteria alone, the fungi and actinomycetes having been largely disregarded, except for occasional demonstrations of their occurrence. Another cause for confusion was the recognition of the mesophilic population alone, as if this population were the one primarily concerned in the decomposition processes. Material taken from hot composts was usually plated out on media favorable for the development of bacteria, and the plates were incubated at temperatures at which only the mesophilic organisms could develop. Under these conditions, the thermophilic population grew to only a very limited extent. Conclusions were frequently reached that the bacterial population originally present in the manure died out in the process of composting, when high temperatures were attained. No consideration was given at all to the changing population. As a result of this, the great majority of plate counts of microorganisms in manures and in composts failed to include most of the organisms primarily concerned in the decomposition processes at high temperatures. When stable manures or plant residues are placed in heaps, nutrient salts added if necessary, and favorable moisture and aeration maintained, the temperature of the compost rapidly rises to 60°–75°C., and even to 80°–85°C. The most important chemical changes are brought about (16) at these temperatures. A knowledge of the nature and activities of the microorganisms active under these conditions is, therefore, of prime importance to recognition of the significance of the processes involved.

The results of Dehérain and Demoussy have been confirmed fully by other investigators. It was brought out in a previous contribution (15) that an increase in temperature, within the range 7° to 37°C., is accompanied by an increase in the rapidity of decomposition, as well as by a change in the chemical nature of the process; at lower temperatures, the lignins were not attacked at all or to only a very limited extent; at higher temperatures, the lignins were attacked more rapidly, although not to the same extent as the other plant constituents, notably the cellulose and hemicelluloses.

EXPERIMENTAL

In a series of preliminary experiments on the decomposition of stable manure and plant residues at higher temperatures, the results of which have already been published (19), it was found that the particular temperature influences

not only the specific nature of the microbiological population but also the rapidity and the type of decomposition. It was shown, for example, that the rapidity of decomposition during the early stages is greater at 65°C. than at 50°C. The latter composts contained an extensive population of fungi belonging largely to the genus *Thermomyces*, similar to the form described by Tsiklinsky (13) and Miehe (8). On the other hand, actinomycetes grew extensively at both temperatures. There was a definite sequence of populations, which varied with the temperature. At 50°C., the fungi appeared first, followed by bacteria which attacked the fungus mycelium, then by actinomycetes. At 65°C., no fungi developed, whereas the bacteria and actinomycetes were most important.

In the following investigations, an attempt was made to determine the influence of a much wider range of temperatures, namely, 28°–75°C., upon the decomposition processes taking place in composts of stable manures. A study was also made of the specific nature of the microbiological populations responsible for the decomposition of the various chemical constituents at the different temperatures and of the sequence of these populations. A comparison was further made of the activities of the soil population and of the thermophilic population of composts upon stable manures and of pure cultures of thermophilic microorganisms.

Methods used

For the study of the chemical changes taking place during the decomposition of manure at different temperatures, the proximate method of analysis was found to be very convenient. This was supplemented by various other analyses, notably that of ammonia and of nitrate, the latter being determined both by colorimetric and reduction methods. For the study of the microbiological population, the plate method and the contact slide or the Rossi-Cholodny methods were employed. The plates were incubated at the same temperatures as the manure. Several media were first tested (19), and two were finally adopted, namely, egg-albumin agar for fungi and actinomycetes and nutrient agar for bacteria. Considerable difficulty was experienced in incubating the plates at 75°C., because the agar liquefied readily. The results reported for this temperature were, therefore, not conclusive. The slides were placed in contact with the manure at the beginning of the experiment and removed at various intervals corresponding to the time of plating. At each sampling date, fresh slides were placed in the compost and allowed to incubate for 2–5 days, in order to obtain a picture of the predominating population at the particular decomposition stages. A detailed summary of the use of the contact slide method for the study of the soil population has recently been published from this laboratory (11). The slides were stained with phenolic rose bengal for 10–15 minutes, over a boiling water bath. The slides were washed, dried, and examined with the microscope, using a 2-mm. apochromatic objective (N. A. 1.30) and 12.5 x compensating oculars. The

immersion oil was added directly to the preparations. A number of fields were examined, and some of the characteristic formations photographed by means of a Bausch and Lomb type H camera. None of the illustrations have been retouched.

Plan of experiment

A mixture of horse manure was prepared, consisting of 10 parts, by weight, of fresh droppings, 1 part of air-dry cut wheat straw, and of 2 parts of fresh urine. These constituents were carefully mixed and distributed into 16 glazed earthenware pots. Each pot received 600 gm. of the moist material. The pots were covered with glass plates and incubated, in quadruplicates, at 28°, 50°, 65°, and 75°C.; the incubators were electrically controlled. In the case of the 75° compost, a constant temperature water bath was used. The fresh manure contained, on a dry basis, 74.1 per cent moisture, 1.58 per cent total nitrogen, and 0.39 per cent ammonia nitrogen. The total fresh material was thus calculated to be 155.4 gm. of dry material per pot. The protein nitrogen reported in the tables was determined by subtracting the water-soluble from the total nitrogen and multiplying the difference by 6.25.

At various intervals, samples of the manure undergoing decomposition at the various temperatures were removed from some of the pots and analyzed for moisture, ash, ammonia, and numbers of microorganisms. At less frequent intervals, proximate chemical analyses were made. The contents of the pots were mixed thoroughly at each sampling. The moisture content was kept as near to 75–80 per cent as possible.

The total amount of every chemical constituent left in the compost was calculated by multiplying the total weight of the residue by the percentage composition. In view of the fact that the latter, as determined by the proximate method of analysis, was always slightly less than 100 per cent, the sum of the weights of the constituents was less than the total residual weight of the compost. By taking into consideration the weights of the samples removed for the various analyses, the results were calculated on the basis of the original total material per individual pot. After 19 days' decomposition, the contents of each two pots were combined into one, so as to prevent rapid drying of the material.

Chemical changes

The results of the proximate chemical analyses of the fresh horse manure and of the manure decomposing at several controlled temperatures are presented in tables 1–5.

Within the first 9 days, the most active decomposition of the manure was found to take place at 65°C., followed by that at 50°C., and the least active, at 75°C. This can be demonstrated, on the one hand, by the reduction in the total weight of dry material, as well as of the cellulose and hemicelluloses, and, on the other hand, by the increase in total protein and by the relative

increase in ash and lignin. It is of particular interest to note that, during this period, the compost kept at 28°C. decomposed only to a limited extent. This was due to the specific nature of the microbiological population, as will be shown later. The relative rate of decomposition of the manure during the early stages has an important bearing upon the conservation of the nitrogen

TABLE 1

Chemical composition of fresh horse manure

CHEMICAL CONSTITUENTS	RELATIVE COMPOSITION	TOTAL CONSTITUENTS PER POT, DRY BASIS
	per cent	*gm.*
Water-soluble organic matter	4.45	6.92
Hemicelluloses	20.55	31.93
Cellulose	30.89	48.00
Lignin	20.46	31.80
Ash	8.92	13.86
Protein, water-insoluble	5.75	8.94
Total	91.02	155.4*

* Actual total dry material.

TABLE 2

Chemical composition of horse manure decomposing at different temperatures for 9 days

CHEMICAL CONSTITUENTS	RELATIVE COMPOSITION				TOTAL CONSTITUENTS, PER POT			
	28°C.	50°C.	65°C.	75°C.	28°C.	50°C.	65°C.	75°C.
	per cent	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*
Water-soluble organic matter	5.16	6.82	5.19	9.16	6.93	8.16	6.09	12.68
Hemicelluloses	19.42	18.64	16.28	18.61	26.06	22.31	19.10	25.76
Cellulose	27.43	28.51	26.60	33.90	36.81	34.13	31.20	46.92
Lignin	20.41	20.46	22.82	18.80	27.39	24.49	26.77	26.02
Ash	9.80	10.80	11.70	9.80	13.15	12.93	13.72	13.56
Protein, water-insoluble	8.38	10.25	12.06	5.63	11.25	12.27	14.15	7.79
Total	90.60	95.48	94.65	95.90	134.2*	119.7*	117.3*	138.4*

* Actual total dry material left.

in the manure. Wherever decomposition was delayed during this stage, losses of nitrogen occurred.

After 19 days' incubation, the total decomposition of the manure was found to be greatest at 50°C., followed by that at 65°C.; it was again least at 75°C. During this period, namely between 9 and 19 days, the manure kept at 28°C. was also attacked rapidly by microorganisms, the actual loss in dry matter being now greatest at this temperature. This is shown by the

reduction, during the second period, of 26.3 gm. dry material per pot, at 28°; at 50°, the reduction was 21.7 gm., and at 65°, only 13 gm.

After 33 days, the total decomposition was still greatest at 50°C., followed now by that at 28°C. The loss in weight was 21.4 gm. at 50°, as compared with 18.2 gm. at 28°, and 11.2 gm. at 65°C. The amount of decomposition

TABLE 3

Chemical composition of horse manure decomposing at different temperatures for 19 days

CHEMICAL CONSTITUENTS	RELATIVE COMPOSITION				TOTAL CONSTITUENTS, PER POT			
	28°C.	50°C.	65°C.	75°C.	28°C.	50°C.	65°C.	75°C.
	per cent	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*
Water-soluble organic matter	5.73	7.08	6.20	7.63	6.08	6.94	6.47	10.36
Hemicelluloses	17.34	14.65	12.74	13.55	18.71	14.36	13.29	18.40
Cellulose	21.12	20.75	26.28	34.18	22.79	20.34	27.41	46.42
Lignin	24.07	23.50	23.72	21.21	25.97	23.03	24.74	28.80
Ash	11.92	13.85	13.07	10.01	12.86	13.57	13.63	13.59
Protein, water-insoluble	12.12	15.31	12.50	6.69	13.08	15.10	13.04	9.08
Total	92.30	95.14	94.51	93.27	107.9*	98.0*	104.3*	135.8*

* Actual total dry material left.

TABLE 4

Chemical composition of horse manure decomposing at different temperatures for 33 days

CHEMICAL CONSTITUENTS	RELATIVE COMPOSITION				TOTAL CONSTITUENTS, PER POT			
	28°C.	50°C.	65°C.	75°C.	28°C.	50°C.	65°C.	75°C.
	per cent	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*
Water-soluble organic matter	6.05	5.58	4.53	9.63	5.43	4.27	4.22	12.95
Hemicelluloses	14.84	11.29	10.84	12.68	13.31	8.65	10.09	17.05
Cellulose	14.47	9.38	19.61	34.48	12.98	7.19	18.26	46.38
Lignin	27.31	25.50	25.70	20.56	24.50	19.53	24.11	27.65
Ash	16.26	19.00	17.25	10.42	14.59	14.55	16.06	14.02
Protein, water-insoluble	13.82	19.57	15.51	7.25	12.40	14.99	14.44	9.75
Total	92.75	90.32	93.44	95.02	89.7*	76.6*	93.1*	134.5*

* Actual total dry material left.

that took place at 75°C. remained very insignificant. After 47 days, total decomposition was still greatest at 50°, followed by that at 28° and 65°; it remained almost at a standstill at 75°. The loss of total dry material, however, was now highest at 65°, namely 8.9 gm., followed by 6.3 gm. at 50°, 5.2 gm. at 28°, and 2.2 gm. at 75°.

The major decomposition of the horse manure which was kept at 75°C. occurred during the first 9 days. It is possible that a large part of the loss at this high temperature was due to the volatilization, during this period, of some of the constituents of the manure. The cellulose was not decomposed at all, the reduction being very insignificant even after 47 days. Only the hemicelluloses were attacked. The small reduction in the lignin content of the manure kept at this temperature was at least partly due to the fact that some of it was brought into aqueous solution as a result of the alkalinity of the compost and the high temperature; this was responsible for the large amount of organic matter in the water extract.

The nature of the microbiological population of the composts kept at the different temperatures can fully explain the chemical changes that occurred. At 75°C., there were no fungi or cellulose-decomposing bacteria. The only

TABLE 5

Chemical composition of horse manure decomposing at different temperatures for 47 days

CHEMICAL CONSTITUENTS	RELATIVE COMPOSITION				TOTAL CONSTITUENTS, PER POT			
	28°C.	50°C.	65°C.	75°C.	28°C.	50°C.	65°C.	75°C.
	per cent	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*
Water-soluble organic matter	5.32	6.97	4.59	6.11	4.50	3.66	3.87	8.08
Hemicelluloses	9.52	7.74	7.53	10.41	8.04	4.92	6.34	13.77
Cellulose	11.99	4.14	17.64	35.10	10.06	2.82	14.85	46.44
Lignin	27.69	25.84	25.13	21.23	23.40	18.17	21.16	28.09
Ash	17.50	21.70	19.30	10.05	14.79	15.26	16.25	13.30
Protein, water-insoluble	15.69	23.50	15.98	8.00	13.26	16.52	13.46	10.58
Total	87.71	89.89	90.17	90.90	84.5*	70.3*	84.2*	132.3*

* Actual total dry material left.

organisms detected by the contact slide method were spore-forming bacteria, largely anaerobic in nature, many belonging to the *Plectridium* type, and capable of attacking sugars and hemicelluloses. It is these bacteria which were chiefly responsible for the reduction of the hemicelluloses in the compost. Since anaerobic bacteria synthesize comparatively little cell substance, there was little increase in the insoluble protein. A detailed study of some of these anaerobic bacteria growing at high temperatures was recently made by Glathe (4). Certain actinomycetes were found at this temperature after long incubation, as will be shown later.

At 65°C., the rapid development of bacteria and of actinomycetes brought about active destruction of the various constituents of the manure. Fungi developed at this temperature to only a very limited extent. The thermophilic bacteria gradually diminished, giving place largely to the thermophilic

actinomycetes. The decomposition of the cellulose at this temperature was carried out by actinomycetes and thermophilic anaerobic bacteria. The latter could readily be demonstrated by the use of a mineral solution containing cellulose as the only source of carbon, the medium being placed in deep tubes. The bacteria developed from the 65° compost in dilutions of 1:20,000, on the basis of moist manure. The number of thermophilic cellulose-decomposing bacteria in the compost was, therefore, about 100,000 per 1 gm. of dry material. A small amount of lignin was decomposed at 65°, but this was less than its decomposition at 50° and about equal to that at 28°C.

The 50°C. compost was at first somewhat slower to decompose than the 65°C. compost. After the first few days, however, the population became much more active at 50°. The organic matter was reduced in 47 days from 141.5 gm. of dry material to 55 gm., a loss of more than 60 per cent. All the constituents underwent active decomposition, but especially the cellulose, 94 per cent of which was decomposed during this period. The decomposition of the hemicelluloses was nearly as great, namely, about 85 per cent. The lignin was reduced from 31.8 gm. to 18.17 gm., a loss of 43 per cent, which was less than the reduction of total organic matter. The proteins increased, both relatively and in total concentration, at the expense of the ammonia in the manure, as a result of the synthesizing activities of the microorganisms. The carbohydrates were thus found to be decomposed more rapidly, and the lignins to be decomposed less rapidly than the total material, while the proteins increased. The active organisms at 50° were largely bacteria, fungi, and actinomycetes. Cellulose decomposition was carried out at this temperature by thermophilic representatives of these three groups of organisms. The number of cellulose-decomposing organisms, as determined by the cellulose tube method, was about 200,000 per 1 gm. of moist compost.

At 28°C., decomposition was at first rather slow, but it became more rapid later, especially when active development of fungi took place. The predominating population in the manure kept at this temperature was the most varied of all, comprising bacteria, fungi, protozoa, nematodes, and a few actinomycetes. The cellulose was decomposed, at this temperature, largely by aerobic bacteria belonging to the *Cytophaga* and *Cellfalcicula* types. These were found extensively in the dilution tubes of 1:20,000 to 1:100,000, in 1 gm. of moist material, thus giving a minimum of 100,000 to 500,000 aerobic cellulose-decomposing bacteria per 1 gm. of dry material. At temperatures lower than 28°C., the rate of decomposition would be still slower, as can be illustrated by the results of a previous experiment (14). At 18°–23°C., the decomposition of horse manure was, in 290 days, about on a level with the decomposition of this compost kept at 28° for 47 days.

Among the various chemical changes taking place in the manure incubated at different temperatures, the transformation of the nitrogen is of particular interest. The results of the analyses of various forms of nitrogen in the composts are presented in table 6. About 22 per cent of the total nitrogen

in the fresh manure was in the form of ammonia. Within the first 2 days of decomposition, the ammonia tended to increase. As soon as active decomposition of the carbohydrates set in, the ammonia was rapidly assimilated by the organisms and transformed into microbial protein. After 5 days, the ammonia was reduced to one-half its original concentration at 65°C., where the decomposition was greatest. In 19 days, only a trace of ammonia was left at 28° and 50°C. At 75°C., the ammonia remained longest, in spite of active volatilization due to the high temperature and alkaline reaction. After 33 days, traces of nitrate appeared at 28°. The amounts of nitrate found in the manure kept at 28° and 50° were considerable after 47 days and increased very rapidly on further incubation. The cellulose and hemicelluloses have now been reduced to minimum concentrations; synthesis of new proteins diminished rapidly. Under these conditions, the nitrogen began

TABLE 6

Influence of temperature upon nitrogen changes in horse manure

Milligrams of nitrogen per pot

INCUBATION	28°C.			50°C.			65°C.			75°C.		
	Total N	NH_3 – N	NO_3 – N	Total N	NH_3 – N	NO_3 – N	Total N	NH_3 – N	NO_3 – N	Total N	NH_3 – N	NO_3 – N
days												
0	2,766	606	0	2,766	606	0	2,766	606	0	2,766	606	0
5	.....	637	0	.....	660	0	.....	300	0	.....	617	0
9	2,161	188	0	2,662	515	0	2,651	176	0	2,201	484	0
19	2,169	Tr.	0	2,509	Tr.	0	2,399	156	0	2,132	352	0
33	2,081	Tr.	Tr.	2,528	Tr.	0	2,439	Tr.	0	2,139	282	0
47	2,197	Tr.	14	2,742	Tr.	7	2,551	143	0	2,157	218	0
61	.....	16	91	.....	14	208	.....	326	11	.....	288	19

to mineralize. Similar conditions prevailed at 65°, but, as the nitrifying bacteria remained inactive at this temperature, the ammonia nitrogen, which disappeared in 33 days, began to accumulate in considerable amounts. Nitrogen liberation was thus found to correspond closely with the processes of secondary decomposition.

When the composts were allowed to incubate for 61 days at the different temperatures, nitrate formation was greatest at 50°C., followed by that at 28°C., with only small amounts of ammonia accumulation. Ammonia formation was greatest at 65° and 75°C. It is interesting to note that small amounts of nitrates were now found also at these temperatures. Whether this nitrate is of biological or of chemical origin remains to be determined.

A graphic summary of the chemical changes taking place in the decomposition of horse manure at different temperatures is presented in figures 1 and 2. Comparison of the rates of decomposition of the cellulose at different temperatures shows that the 50° curve, although at the start somewhat above

that of 65°, soon crosses it and is the steepest of the whole set. The 75° curve is virtually a straight line, indicating no decomposition. The 65° curve is the first to drop sharply, but it soon flattens out, giving way, first to the 50° curve, then to the 28° curve. The curves showing changes in ash content and protein content are almost parallel with the curves for total

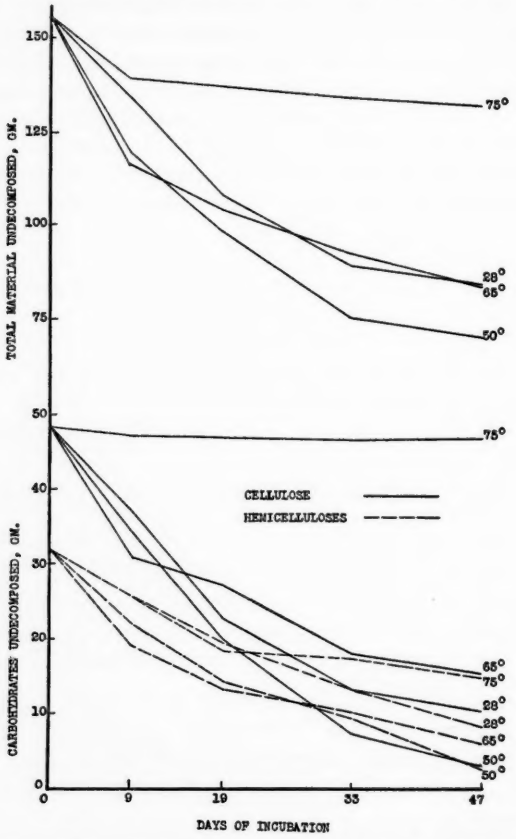


FIG. 1. INFLUENCE OF TEMPERATURE (°C.) UPON THE COURSE OF DECOMPOSITION OF HORSE MANURE, AS MEASURED BY LOSS OF TOTAL DRY MATERIAL AND CARBOHYDRATES

decomposition, except for minor discrepancies between the 28° and 65° curves; these coincide at the end of the decomposition period in the case of loss in total material and increase in protein, but remain parallel for the ash content.

These results bear out emphatically the synthesizing activities of the microorganisms during the decomposition of the manure. The lignin curves bring out certain important results, namely that greater accumulation of

lignin took place at 28° than at 50° and 65°, in spite of the fact that there was less total decomposition at 28° than at 50°. This is due entirely to the fact that the thermophilic fungi and actinomycetes are more capable of decomposing the lignin than are the mesophilic organisms. These results thus confirm earlier studies (15) on the decomposition of manures and plant materials at lower temperatures, where the fact was brought out that the lower the temperature the greater is the resistance of the lignin to decomposition, thus resulting in its accumulation.

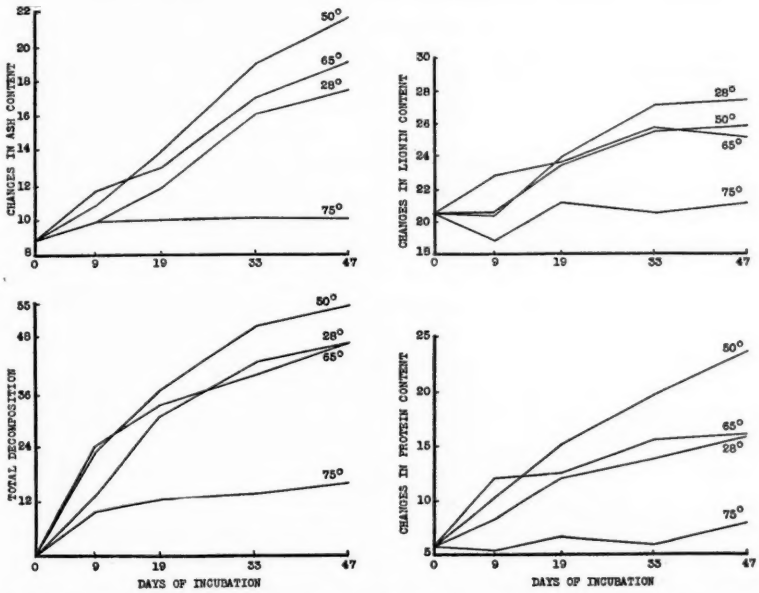


FIG. 2. CHEMICAL CHANGES IN HORSE MANURE DECOMPOSING AT DIFFERENT TEMPERATURES (°C.), ON PERCENTAGE BASIS

Micropopulation changes

The results obtained, by the plate method, on the numerical changes taking place in composts of horse manure decomposing at different temperatures are presented in table 7. At 28°C., the bacteria predominated, the fungi began to develop late in the incubation period, and the number of actinomycetes was very limited. At 50°C., the bacteria persisted for several days, then rapidly diminished, their place being taken by fungi and actinomycetes. At 65°C., the bacteria (except for specific anaerobic forms) and the fungi disappeared rapidly, their place being taken largely by actinomycetes. At 75°C., certain spore-forming bacteria were the only surviving organisms.

The existence of a definite correlation between the plate method and the contact slide method has been indicated in a number of investigations including the study of composting manure (6). This has also been fully confirmed by the results of the present investigations. Plates 1–7 illustrate graphically the nature of the microbiological populations responsible for the decomposition of manure at different temperatures. They further em-

TABLE 7

Influence of temperature upon the development of microorganisms in manure composts

Per gram of moist compost

TEMPERATURE OF INCUBATION	PERIOD OF INCUBATION	BACTERIA	ACTINOMYCETES	FUNGI
°C.	*days*	*millions*	*millions*	*thousands*
28	0	1,600	0.2	200
	2	14,000*	.....	0
	5	.....	.....	.....
	8	175*	.....	.....
	21	85*	.....	11,000
	39	50*	.....	600
50	0	1,600	0.2	200
	2	100*	.....	.....
	5	850	150	.....
	8	1,000	1,000	.....
	21	Few	14	2,000
	39	0	6.4	1,000
65	0	1,600	0.2	200
	2	100*	.....	0
	5	.....	2	0
	8	.....	106	0
	21	.....	2.5	0
	39	.....	7.6	0
75	0	1,600	0.2	200
	8	3.5	0	0
	21	2.0	0	0

* Including actinomycetes.

phasize the changes in these populations with the course of decomposition. Some of the most striking results are the following:

At 28°C. (plates 1, 2), the bacteria, fungus, and animal populations were all characteristic of the compost. The bacteria were the first to develop, and they remained predominant throughout the whole compost period, thus justifying the general emphasis upon the rôle of bacteria in manure, when the methods used favor the development of mesophilic organisms only. The bacteria were represented by different types, comprising nonspore-forming and spore-forming rods, varying considerably in size, as well as small and

large cocci, chains of bacteria, individual cells, and colonies ranging from masses of cells to zoogleal formations. Many of the rods were definitely curved and closely resembled *Cytophaga*, an organism found abundantly in the cellulose tubes. The fungus population was represented at 28° by Phycomycetes (fig. 13), Ascomycetes (fig. 10), and Fungi Imperfecti (figs. 11, 12). Certain genera, like *Zygorhynchus*, *Humicola*, *Trichothecium*, *Cephalosporium*, *Alternaria*, and others, could readily be recognized from the stained preparations. The animal population was represented by protozoa, largely flagellates and ciliates (figs. 14, 15), and by nematodes (fig. 16). Among the most striking characteristics of this population one may note: (*a*) the almost complete absence of actinomycetes at this temperature, during the period of study; (*b*) the heterogeneous nature of the population; (*c*) the predominance of bacteria and animal forms. There was thus an excellent correlation between plate and contact slide methods.

At 50°C. (plates 3, 4), the microbiological population was distinctly different from that of 28°. Here as well, bacteria and fungi appeared rapidly, but the actinomycetes became established early in the decomposition period. The fungi were largely represented by the *Thermomyces* (fig. 24) and the *Monilia* or *Oidium* (fig. 25) groups. The bacteria and actinomycetes persisted throughout the decomposition process, but the fungi tended later to disappear, because of the rapid destruction of their mycelium by the other organisms. The most characteristic features of the 50° population are the following: (*a*) lack of animal forms, (*b*) abundance of actinomycetes, (*c*) active thermophilic fungi, followed by bacteria and actinomycetes, (*d*) specific bacterial types.

At 65°C. (plates 5, 6), bacteria and actinomycetes were the first to appear in very great abundance. No animal forms were ever observed. Fungi either were absent altogether or were present only seldom, namely, in the 2-day-old preparations. In view of the fact that the fungi were completely absent on the plates, their rare appearance on the slides may be ascribed to possible growth at the edges of the compost. The bacteria were represented by a number of larger and smaller rod-shaped organisms, both spore-forming and nonspore-forming. The spore-formers included thermophilic cellulose-decomposing bacteria. Some of the bacteria occurred in colonies. The actinomycetes were represented by two genera, namely, *Actinomyces* and *Micromonospora*, each comprising at least three distinct species described in detail elsewhere (19). With the advance of the decomposition, the *Micromonospora* types dominated completely all the fields. Very often certain spherical bodies varying in size appeared, as shown in figure 47. Bodies of this type have frequently been observed by others in cultures of actinomycetes and sometimes have been spoken of as "chlamydospores" or "yeast-like growths." The most important characteristics of the 65° population can be summarized as follows: (*a*) predominance of an extensive population of actinomycetes, (*b*) the particular abundance of the genus *Micromonospora*,

represented by a number of different species (figs. 36–42, 44–46), (*c*) complete absence of animal forms and almost complete absence of fungi, (*d*) abundance of spore-forming bacteria.

At 75°C. (plate 7), the population consisted almost entirely of spore-forming bacteria. In time, the vegetative rods disappeared, and only the masses of spores were left. Fungi and animal forms were lacking entirely. Actinomycetes developed at a later period and chiefly at the surface of the compost, where the temperature may have been somewhat lower than 75°. The microscopic picture fully confirmed the quantitative results obtained by the plate method. This picture is much more dependable, however, because of the difficulty experienced in incubating the plates at 75°. It is to be recalled further that no cellulose-decomposing organisms developed at this temperature, a fact which accounted for the quantitative accumulation of the cellulose.

A number of other important observations can be noted from the study of the plates. Figures 12 and 26 illustrate the decomposition of fungus mycelium by bacteria, and figure 28 illustrates the attack of actinomycetes upon fungus mycelium. This phenomenon was first observed in this laboratory some 15 years ago by the use of the suspended drop culture method (17, 18), and by others, by the use of the contact slide method. This fully explains the frequently observed sequence of microbial populations, the fungi developing first, later followed by bacteria and actinomycetes. The concept of specific response of microorganisms to organic nutrients observed previously (18, 7, 20), can now be enlarged to include conditions of decomposition. Among the latter, the specific response of the soil population to soil reaction has already been noted by many investigators. The particular response to temperature, as brought out in these investigations, is noteworthy in this connection.

SUMMARY

One of the major problems in the composting of stable manures is the control of the rapidity of decomposition of the organic constituents and the conservation of the soluble nutrient elements essential for plant growth, especially the nitrogen.

The temperature of the compost is one of the most important factors in controlling the rapidity of the decomposition of the manure and the conservation of the nitrogen. This is accomplished through the control of the microbiological population which is concerned in the decomposition processes.

The most rapid decomposition of horse manure set in at a temperature of 65°C., followed by that at 50°C. After the first stages of rapid decomposition, the process was found to proceed more rapidly at 50° than at 65°C.

A temperature of 75°C. was found to be unfavorable to biological decomposition of the manure. Only the hemicelluloses were decomposed to any considerable extent. The cellulose was not attacked at all, the increase in proteins was limited, and only a part of the lignin was brought into solution.

At 28°C., there was a considerable delay before active decomposition set in, but after a lapse of 9 or 10 days, the manure began to decompose rapidly, as a result of the development of an extensive microbiological population.

The microorganisms concerned in the decomposition of manure at different temperatures were found to have the following characteristics:

At 75°C., the animal population and the fungi were completely repressed. Actinomycetes appeared only seldom, at the surface of the compost. Only certain types of bacteria were active, belonging largely to the spore-forming, hemicellulose-decomposing types, many of them plectridia.

At 65°C., the bacteria and actinomycetes were chiefly concerned in the decomposition process. Fungi appeared only seldom, and animal forms were absent. The first two groups were represented by a number of characteristic thermophilic groups. After a certain period, the bacteria were gradually reduced and the actinomycetes became the predominant organisms. The thermophilic actinomycetes are limited to very few species, but comprised several genera.

At 50°C., certain thermophilic fungi were very active, in addition to the bacteria and actinomycetes. This selective population, in which fungi and actinomycetes played the predominant rôle, was responsible for the most rapid decomposition of the manure. Among the fungi, one form belonging to a group of organisms, frequently described as *Monotospora*, *Sepedonium*, *Acremoniella*, and *Thermomyces* was found to be most abundant. The actinomycetes were similar to those developing at 65°C.

Lower temperatures, as typified by 28°C., gave rise to a highly heterogeneous population. Bacteria, fungi, actinomycetes, protozoa, and nematodes were well represented by a great variety of forms. A few days elapsed before certain active types became established, a fact which accounts for the delay in the rapidity of the decomposition process at this temperature.

No organism capable of bringing about cellulose decomposition was active at 75°C. At 65°C., thermophilic anaerobic bacteria were largely concerned in the destruction of the cellulose, followed by certain actinomycetes. At 50°C., thermophilic fungi and actinomycetes played the most important rôle in decomposing the cellulose in the manure. At 28°C., aerobic bacteria, belonging to the *Cytophaga* and other groups, were most active.

The nitrogen changes in the manure composted at different temperatures were most significant: Under conditions of active decomposition, the soluble forms were rapidly transformed into microbial cell substance, accompanying the decomposition of the carbohydrates. When the latter were reduced to a certain minimum, the proteins, both originally present in the manure and freshly synthesized by the microorganisms, began to decompose, liberating the nitrogen in a mineralized form.

Nitrification took place only at 28° and 50°C., soon after the rapid decomposition phases were completed, namely, after 33 days. At 65°C., very little nitrate was formed, and the ammonia which resulted from the secondary decomposition processes accumulated.

Nitrogen was conserved in the manure only when immediate decomposition set in. This resulted in a rapid breakdown of the carbohydrates and the transformation of the soluble nitrogen into insoluble forms. Whenever

decomposition was delayed, either because of too low or too high temperatures, losses of the volatile forms of nitrogen occurred.

REFERENCES

(1) CONN, H. J., AND COLLISON, R. C. 1922 Fermentation and preservation of manure: I. A study of certain bacteria involved in the ammonification of manure. N. Y. Agr. Exp. Sta. Bul. 494.

(2) DEHÉRAIN, P. P. 1902 Traité de Chimie Agricole, ed. 2. Paris.

(3) DEHÉRAIN, P. P., AND DEMOUSSY, E. 1896 Sur l'oxydation de la matière organique du sol. *Ann. Agron.* 22: 305–337.

(4) GLATHE, H. 1934 Über die Rotte des Stalldüngers unter besonderer Berücksichtigung der Anaeroben-Flora. *Centbl. Bakt.* (II) 91: 65–98.

(5) HÉBERT, A. 1893 Farm manure. *Exp. Sta. Rec.* 5: 141–158.

(6) HULPOI, N., AND GLATHE, H. 1938 Die Bedeutung der Stalldüngerrotte für den Boden und die Ernteerträge. *Centbl. Bakt.* (II) 98: 1–32.

(7) KUBIENA, W., AND RENN, C. E. 1935 Micropedological studies of the influence of different organic compounds upon the microflora of the soil. *Centbl. Bakt.* (II) 91: 267–292.

(8) MIEHE, H. 1907 Die Selbsterhitzung des Heues. G. Fischer, Jena.

(9) RUSCHMANN, G. 1927–28 Vergleichende biologische und chemische Untersuchungen an Stalldüngersorten. *Centbl. Bakt.*, (II) 70: 214–260, 383–411 (1927); 72: 192–236 (1927); 73: 179–206 (1928).

(10) RUSSELL, E. J., AND RICHARDS, E. H. 1917 The changes taking place during the storage of farmyard manure. *Jour. Agr. Sci.* 8: 495–563.

(11) STARKEY, R. L. 1938 Some influences of the development of higher plants upon the microörganisms in the soil: VI. Microscopic examination of the rhizosphere. *Soil Sci.* 45: 207–249.

(12) TOVBORG-JENSEN, S. 1928–29 Investigations on ammonia evaporation and nitrogen loss in spreading liquid manure. Statens Forsøgsvirks. Plantekult. Beret. 211 (1928); 221 (1929).

(13) TSIKLINSKY, P. 1899 Sur les mucedinées thermophiles. *Ann. Inst. Pasteur* 13: 500–505.

(14) WAKSMAN, S. A., AND DIEHM, R. A. 1929 Chemical and microbiological principles underlying the transformation of organic matter in stable manure in the soil. *Jour. Amer. Soc. Agron.* 21: 795–809.

(15) WAKSMAN, S. A., AND GERRETSEN, F. C. 1931 Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microörganisms. *Ecology* 12: 33–60.

(16) WAKSMAN, S. A., AND NISSEN, W. 1932 On the nutrition of the cultivated mushroom, *Agaricus campestris*, and the chemical changes brought about by this organism in the manure compost. *Amer. Jour. Bot.* 19: 514–537.

(17) WAKSMAN, S. A., AND SKINNER, C. E. 1926 Microörganisms concerned in the decomposition of cellulose in the soil. *Jour. Bact.* 12: 57–84.

(18) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, actinomycetes, and bacteria in the soil. *Soil Sci.* 17: 373–378.

(19) WAKSMAN, S. A., UMBREIT, W. W., AND CORDON, T. C. 1939 Thermophilic actinomycetes and fungi in soils and in composts. *Soil Sci.* 47: 37–61.

(20) ZIEMIECKA, J. 1935 The use of a modified Rossi-Cholodny technic for studying the organisms that decompose certain organic compounds in soil. *Centbl. Bakt.* (II) 91: 379–394.

PLATES

PLATE 1

MICROBIOLOGICAL POPULATION ACTIVE IN THE DECOMPOSITION OF HORSE MANURE AT 28°C.

This population consists predominantly of bacteria, especially in the early stages

FIGS. 1–3. One-day-old compost, showing different types of bacteria, nonspore-forming and spore-forming, singly, in chains, and in colonies. × 695.

FIGS. 4–5. Two-day-old compost, showing predominance of heavy rods. × 695.

FIG. 6. Four-day-old compost, showing heterogeneous bacterial population and protozoan cysts. × 695.

FIG. 7. Nine-day-old compost, showing large masses of bacteria and filaments of actinomycetes. × 695.

FIG. 8. Thirty-four-day-old compost, showing different types of bacteria distributed singly and in colonies. × 695.

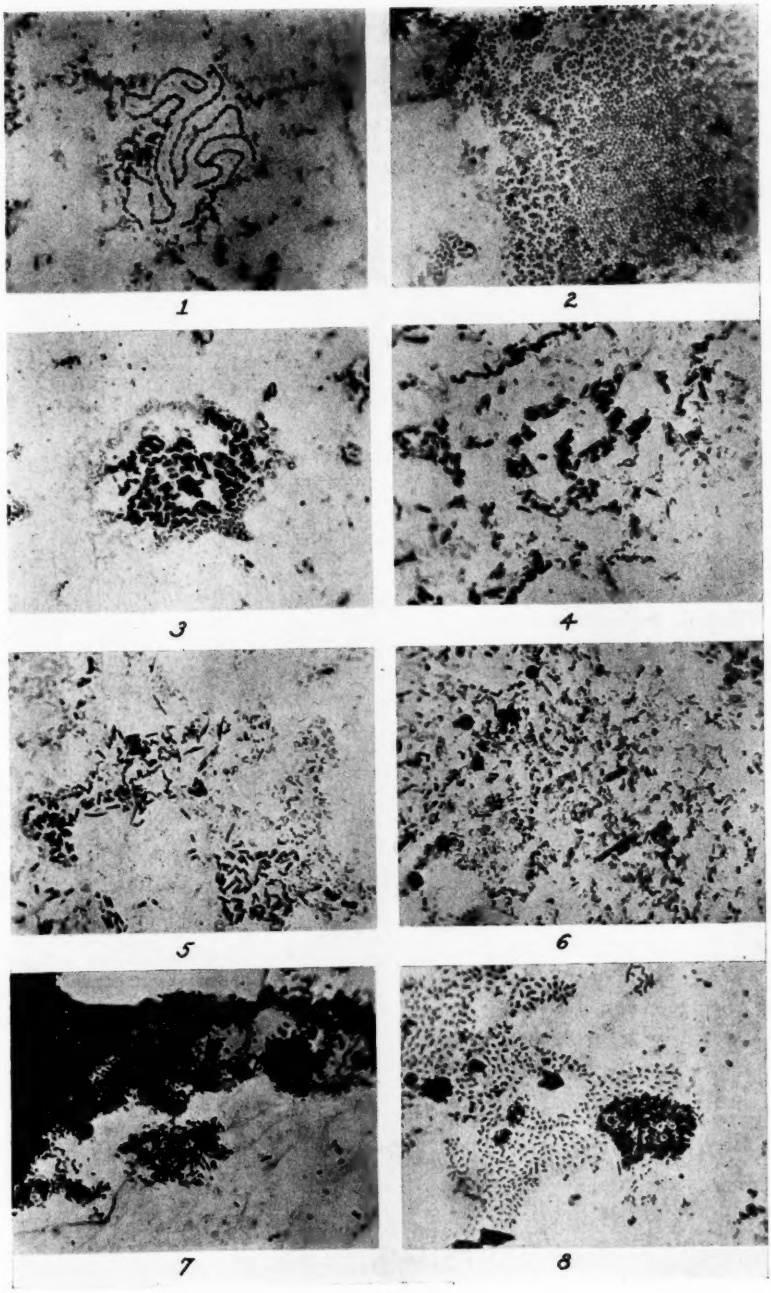

1
2
3
4
5
6
7
8

PLATE 2

OTHER MEMBERS OF THE MICROBIOLOGICAL POPULATION OF COMPOSTS KEPT AT 28°C.

FIG. 9. Fifteen-day-old compost. Fungus filaments and protozoan cysts. × 325.

FIG. 10. Fifteen-day-old compost. Fungus filaments and perithecea-like bodies. × 74.

FIG. 11. Nineteen-day-old compost. Fungus mycelium with single cylindrical-shaped cells, borne on side branches. × 74.

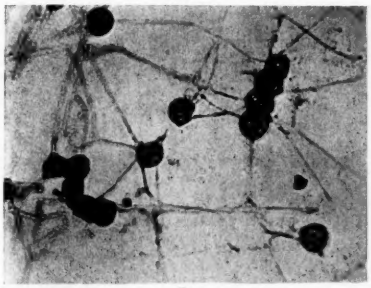
FIG. 12. Thirty-four-day-old compost. *Cephalothecium*-like organism. × 695.

FIG. 13. Thirty-four-day-old compost. Mycelium and sexual spores of *Zygorhynchus*. × 325.

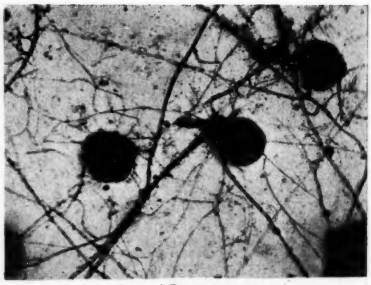
FIG. 14. Ciliates found in 34- and 40-day-old composts. × 325.

FIG. 15. Ciliates found in 34- and 40-day-old composts. × 695.

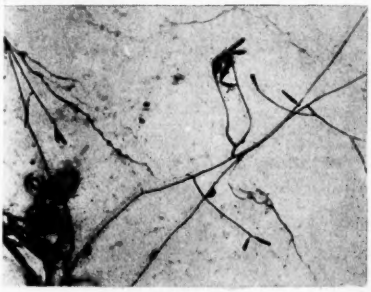
FIG. 16. Nematodes in 15-day-old compost. × 74.



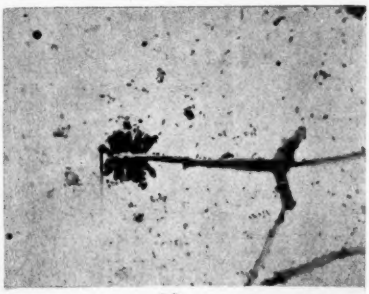
9



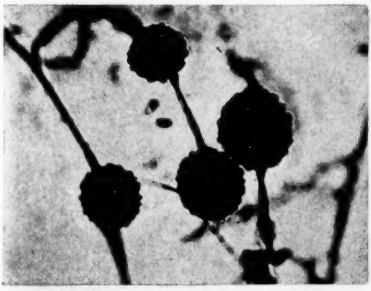
10



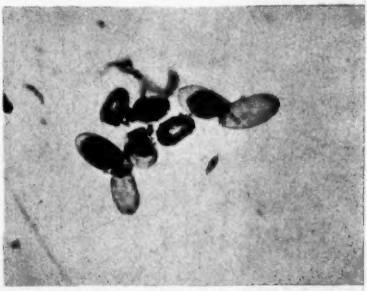
11



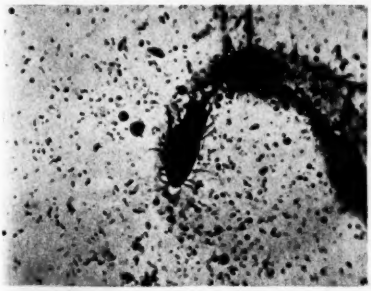
12



13



14



15

16

PLATE 3

MICROBIOLOGICAL POPULATION ACTIVE IN THE DECOMPOSITION OF HORSE MANURE AT 50°C.

FIG. 17. One-day-old compost, showing chains and masses of spore-forming bacteria. × 695.

FIGS. 18–19. One-day-old compost, showing extensive development of two types of actinomycetes, namely *Actinomyces* and *Micromonospora*. × 695.

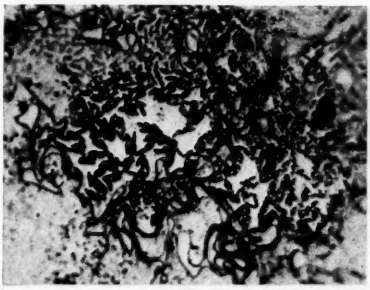
FIG. 20. Two-day-old compost. Extensive bacterial development accompanied by fungus spores. × 695.

FIG. 21. Two-day-old compost, showing spherical bodies, accompanying rod-shaped bacteria. × 695.

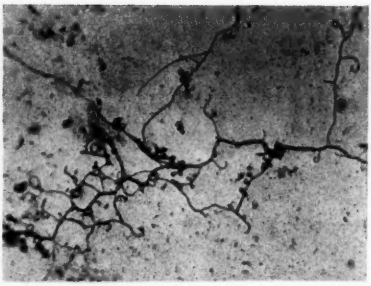
FIG. 22. Seven-day-old compost, showing actinomycetes, spherical bodies and protozoan cysts (?). × 695.

FIG. 23. Seven-day-old compost, showing attack upon plant fiber by bacteria followed by protozoa (?). × 695.

FIG. 24. Extensive development of *Thermomyces* in 15-day-old compost. × 74.

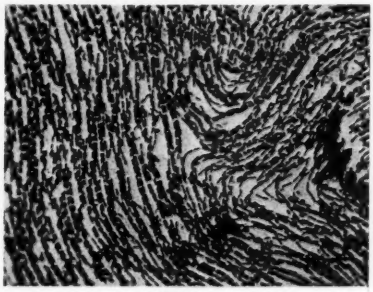


17

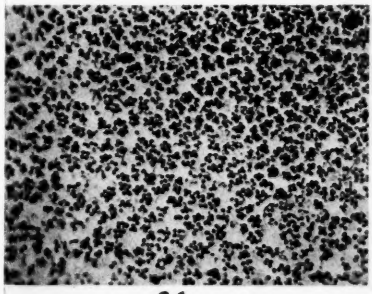


18

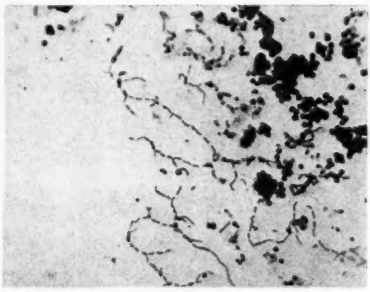
19



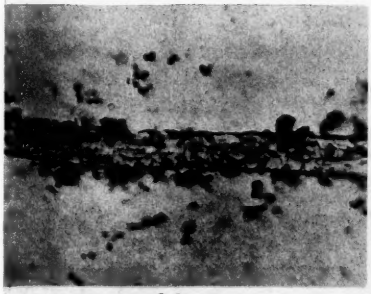
20



21



22



23

24

PLATE 4

MICROBIOLOGICAL POPULATION IN COMPOSTS OF HORSE MANURE AT 50°C.

FIG. 25. Nineteen-day-old compost, showing *Oidium*-like fungus and bacterial spores. × 695.

FIG. 26. Nineteen-day-old compost, showing chains of bacteria accompanying fungus mycelium. × 695.

FIG. 27. Twenty-two-day-old compost, showing development of *Actinomyces thermophilus*. × 695.

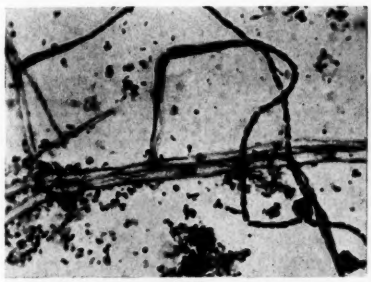
FIG. 28. Thirty-four-day-old compost, showing *Actinomyces thermofuscus* and *Oidium*-like fungus. × 695.

FIG. 29. Forty-day-old compost, showing bacterial colonies, individual bacterial cells, and bacterial spores. × 695.

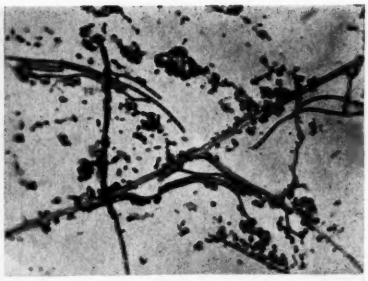
FIG. 30. Four-day-old compost, showing spores of fungi (?), filaments of actinomycetes, *Micromonospora* spores, and coccoid cells. × 695.

FIG. 31. Seven-day-old compost showing spores of fungi (?), bacterial cells, and *Micromonospora* spores. × 695.

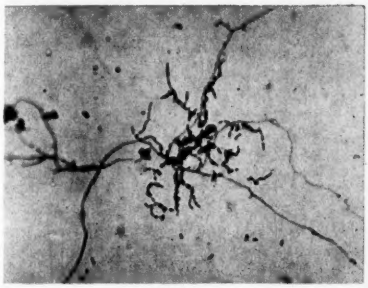
FIG. 32. Forty-day-old compost, showing large numbers of bacterial spores, protozoan cysts, and filaments of actinomycetes. × 695.



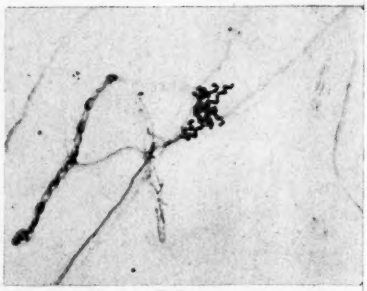
25



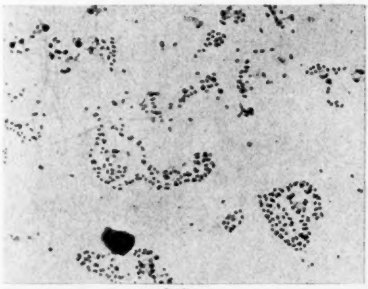
26



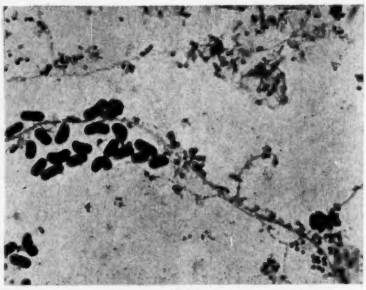
27



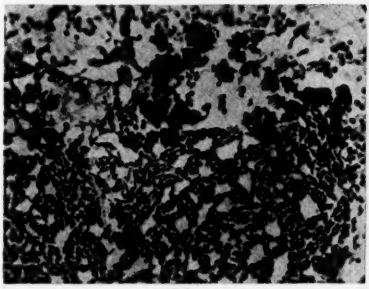
28



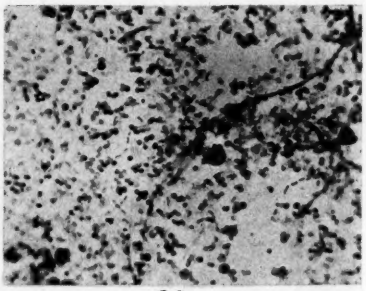
29



30



31



32

PLATE 5

MICROBIOLOGICAL POPULATION OF COMPOSTS OF HORSE MANURE KEPT AT 65°C.

FIG. 33. Twenty-four-hour-old compost, showing long rod-shaped, spore-forming bacteria. × 695.

FIG. 34. Two-day-old compost, showing chains of spore-forming bacteria and more lightly stained masses of bacterial spores. × 695.

FIG. 35. Two-day-old compost, showing short and broad bacteria, thin rod-shaped bacteria, and masses of spores. × 695.

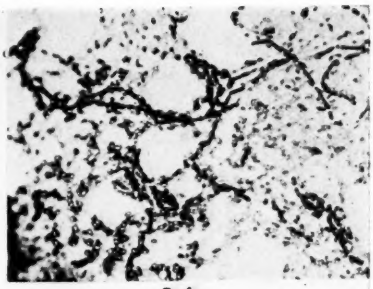
FIG. 36. Two-day-old compost, showing abundance of *Micromonospora vulgaris*. × 695.

FIG. 37–38. Four-day-old compost, showing masses of *Micromonospora fusca*. × 695.

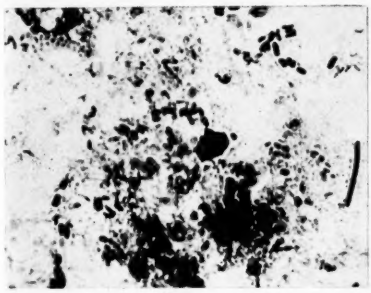
FIG. 39. Five-day-old compost, showing a new type of *Micromonospora* related to *M. chalceae*. × 695.

FIG. 40. Seven-day-old compost, showing *Micromonospora chalceae*. × 695.

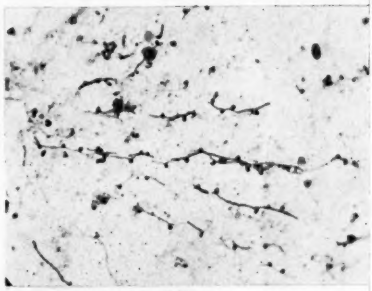
33



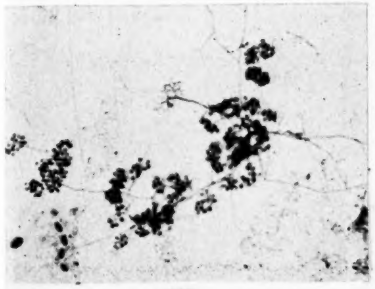
34



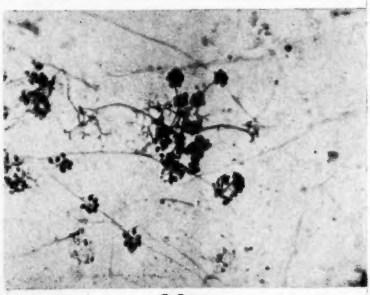
35



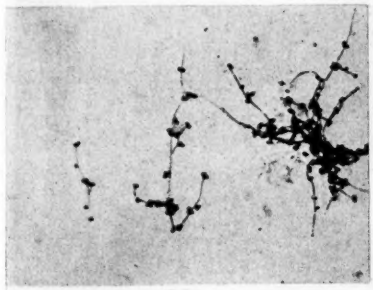
36



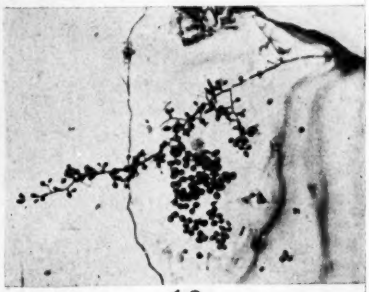
37



38



39



40

PLATE 6

MICROBIOLOGICAL POPULATION OF COMPOSTS OF HORSE MANURE KEPT AT 65°C.

FIG. 41. Nineteen-day-old compost, showing fragmented mycelium and spores of actinomycetes. × 695.

FIG. 42. Nine-day-old compost, showing masses of spores of *Micromonospora*. × 695.

FIG. 43. Fifteen-day-old compost, showing *Actinomyces thermofuscus*. × 695.

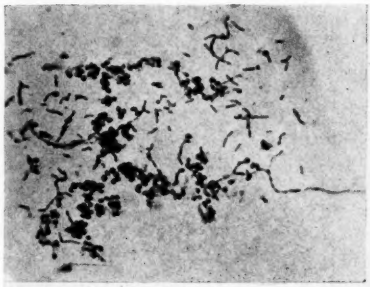
FIG. 44. Nineteen-day-old compost, showing *Micromonospora vulgaris*. × 695.

FIG. 45. Twenty-two-day old compost, showing *Micromonospora chalceae*. × 695.

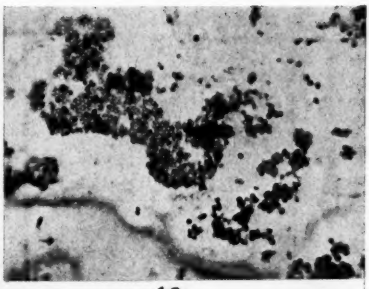
FIG. 46. Twenty-two-day-old compost, showing another preparation of *Micromonospora chalceae*. × 695.

FIG. 47. Thirty-four-day-old compost, showing masses of *Micromonospora* spores and larger bodies, of an unknown nature. × 695.

FIG. 48. Forty-day-old compost, showing masses of bacteria, various bacterial spores, and *Micromonospora* spores. × 695.

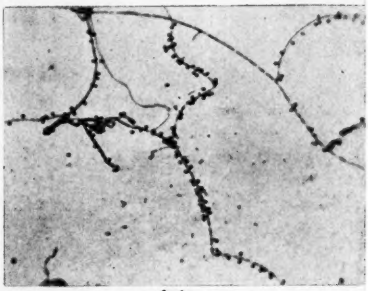


41

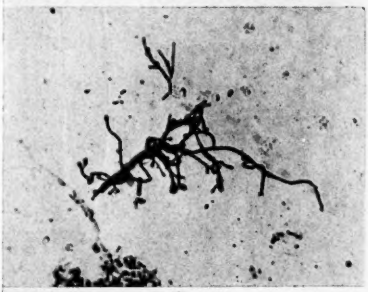


42

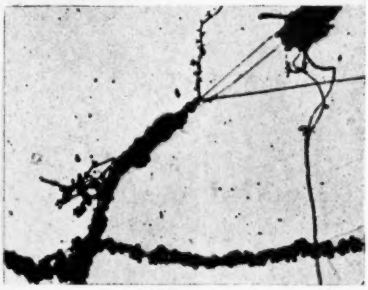
43



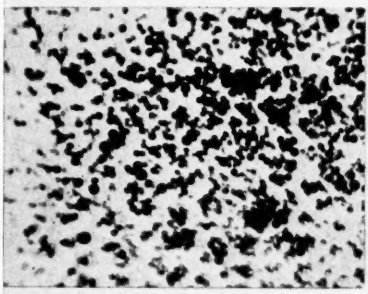
44



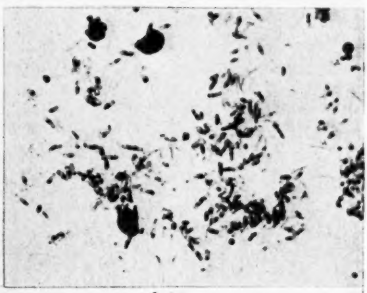
45



46



47



48

PLATE 7

MICROBIOLOGICAL POPULATION OF HORSE MANURE KEPT AT 75°C.

FIG. 49. Twenty-four-hour-old compost, showing several types of rod-shaped bacteria. × 695.

FIG. 50. Four-day-old compost, showing two distinct types of bacteria, short curved rods and long chain-forming rods. × 695.

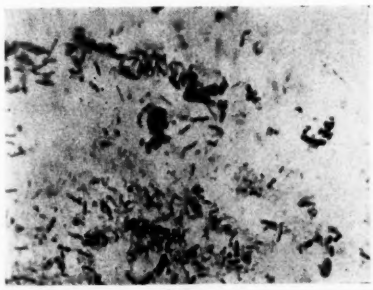
FIG. 51. Five-day-old compost, showing predominance of long, spore-forming rods of the *Plectridium* type, with a number of short rods. × 695.

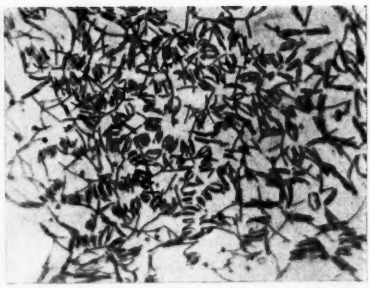
FIG. 52. Fifteen-day-old compost, showing granulation of long rods. × 695.

FIG. 53. Fifteen-day-old compost, showing abundance of bacterial spores and few vegetative cells. × 695.

FIG. 54. Another 15-day-old compost preparation, showing heavy rods and *Micromonospora* mycelium. × 695.

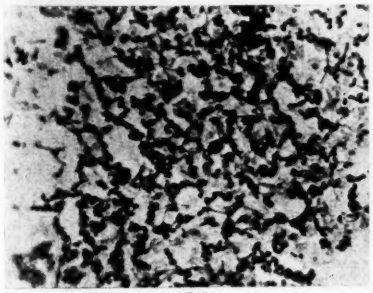
FIG. 55. Forty-day-old preparation, showing mycelium and spores of *Micromonospora vulgaris*. × 695.

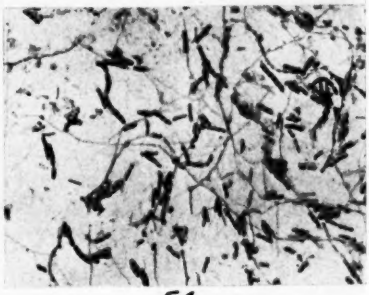

49

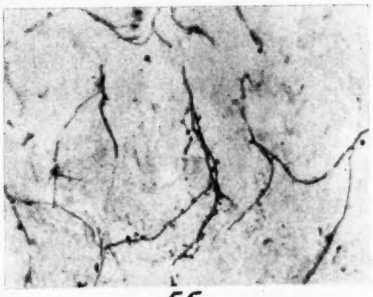

50

51

52


53


54


55

EFFECT OF PHOSPHATES ON NITRIFYING CAPACITY OF SOILS

G. S. FRAPS AND A. J. STERGES

Texas Agricultural Experiment Station

Received for publication July 14, 1938

Previous work on nitrification (8) has shown that the low nitrifying capacity of many soils can be increased considerably by additions of calcium carbonate or of organisms by means of inoculating liquid containing nitrifying bacteria, or by both. Of 171 samples of soil examined, however, 32 nitrified less than 60 per cent of the added ammonia even though nitrifying organisms and calcium carbonate were added. Work was therefore begun to ascertain what other additions would increase nitrification in such soils. Additions of phosphates were found to be effective on many of these soils. Previous work (9) showed that dicalcium phosphate was not sufficiently basic to increase nitrification in soils in which nitrification was increased by calcium carbonate, but in the soils here discussed dicalcium phosphate or other phosphates increased nitrification in the presence of calcium carbonate, indicating that phosphoric acid was needed. This paper presents results obtained in a study of the effect of additions of phosphates upon nitrification in a number of such soils.

EXPERIMENTAL

The soils selected were those in which nitrification was not complete alone or with additions of calcium carbonate and inoculating liquid. The nitrification work was done by the procedure already described (7). In the general procedure, 100 gm. of soil was mixed with 1 gm. of calcium carbonate, the phosphate, if used, 5 cc. of a solution containing 0.05 gm. nitrogen in the form of ammonium sulfate, and 10 cc. of inoculating liquid from an actively nitrifying soil. Water equal to 50 per cent of the moisture-holding capacity of the soil was then added, and the soil was mixed by cutting in with a spatula. The mixtures were transferred to 150-cc. beakers and kept at 35°C., water being added twice a week to restore the loss in weight. After 28 days, the nitrates were estimated by the phenol disulfonic acid method and the nitrites by the alpha-naphthylamine method already described (7). The nitric nitrogen as given here includes also the nitrite nitrogen, if any. The amount of the latter was usually small.

Table 1 gives the results of an experiment in which additions of 0.5 gm. monocalcium phosphate alone and also with 1 gm. of calcium carbonate were made to 100 gm. of soil. The monocalcium phosphate alone increased the

nitrification in 4 of the 18 soils and decreased the nitrification or produced no nitrification in the remaining 14 soils. Nitrification with monocalcium phosphate and calcium carbonate together was greater than with the calcium carbonate alone in 16 soils, showing the need of phosphate for increased nitrification in these particular soils. Nitrite nitrogen to the extent of 82 p.p.m. was found in soil 39699, 50 p.p.m. in 43402, and 130 in 44350, when calcium carbonate was added, but not any in the other cultures, including those which received phosphate. Since the presence of calcium carbonate as well as the phosphate was required on most of these soils, additions of calcium carbonate were made in subsequent experiments.

TABLE 1

Nitric nitrogen production in soils as affected by monocalcium phosphate

SAMPLE NUMBER	SOIL TYPE	DEPTH	NO $CaCO_3$		WITH $CaCO_3$		
			$-PO_4$	$+PO_4$	$-PO_4$	$+PO_4$	Increase due to PO_4
		inches	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
37293	Quanah fine sand	7–19	0	0	152	258	106
37294	Miles fine sand	19–31	28	0	329	303	0
37653	Ruston fine sandy loam	7–19	0	0	322	480	158
39682	Duval fine sandy loam	12–30	37	1	374	456	82
39689	Pryor clay loam	6–24	360	406	338	396	58
39699	Webb fine sandy loam	15–42	0	0	334	482	148
39882	Wilson clay loam	7–19	0	0	255	405	150
39884	Wilson very fine sandy loam	10–19	80	0	387	485	98
41177	Houston black clay	7–19	439	451	445	475	30
41183	Sumter clay	7–19	322	450	246	438	192
41185	Bell clay	7–19	464	235	479	482	3
43387	Leaf fine sandy loam	3–7	0	0	185	228	43
43392	Tabor fine sandy loam	7–18	8	0	312	247	0
43393	Tabor fine sandy loam	19–36	0	0	269	461	192
43397	Lufkin fine sandy loam	7–19	1	0	405	528	123
43400	Wilson very fine sandy loam	7–19	80	0	395	479	84
43402	Crockett very fine sandy loam	7–13	0	0	237	353	116
44350	Webb fine sandy loam	20–24	346	407	302	420	118

The results of another series of experiments in which 1 gm. of dicalcium phosphate was added to 100 gm. of soil are given in table 2. Since it was thought possible that the number of organisms might have an effect, these results were compared with those of a similar series of experiments in which the addition of inoculating liquid was 5 cc. The 10 cc. of inoculating liquid increased the nitrification over that of the 5 cc. in some soils and failed to increase it in others. In each series, nitrification in 29 of the 32 soils was increased by the dicalcium phosphate.

At the end of the experiment, nitrite nitrogen was present in 13 of the 32 cultures which received 5 cc. of inoculating liquid without phosphate and in

10 of those which received 10 cc., in 3 of those to which dicalcium phosphate was added with 5 cc. of inoculating liquid and in 3 with 10 cc. It is thus

TABLE 2

Nitric nitrogen production in soils as affected by dicalcium phosphate

SAMPLE NUMBER	SOIL TYPE	DEPTH	5 CC. INOCULATING LIQUID			10 CC. INOCULATING LIQUID		
			$-PO_4$	$+PO_4$	Increase due to PO_4	$-PO_4$	$+PO_4$	Increase due to PO_4
		inches	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
35109	Vernon clay	7–19	225	400	175	265	410	145
35112	Miles fine sand, shallow phase	0–7	184	249	65	206	283	77
35113		7–24	214	294	80	228	288	60
35114		24–36	393	550	157	247	550	303
35168	Norfolk fine sand	7–36+	125	184	59	130	200	70
35169		0–7	240	256	16	256	281	25
35171	Falls fine sandy loam	7–20	150	500	350	190	563	373
35172	Susquehanna fine sandy loam	0–7	278	291	13	274	286	12
35173	Crockett fine sandy loam	30–36	23	500	477	51	525	474
35178		20–36+	188	575	387	400	550	150
35183	Sumter clay	7–36+	422	600	178	422	588	166
35190	Houston black clay	24–36+	179	500	321	331	550	219
36369	Miles fine sandy loam	0–7	277	288	11	261	330	42
36487	Refugio loamy fine sand	0–7	255	356	101	255	370	115
36488		7–10	220	233	13	235	265	32
36496	Victoria clay	7–24	337	400	63	410	440	30
37294	Miles fine sand	19–31	390	481	91	377	488	111
37652	Ruston fine sandy loam	0–7	220	287	67	243	312	69
37653		7–19	380	512	132	410	512	102
39682	Duval fine sandy loam	12–30	462	525	63	462	550	25
39686	Pryor clay	0–30	100	230	130	148	312	164
39687		30–84	57	235	178	96	293	197
39689	Pryor clay loam	6–24	362	400	38	410	450	40
39699	Webb fine sandy loam	15–42	328	525	197	368	525	157
39880	Sumter clay	7–19	97	525	428	249	462	213
39882	Wilson clay loam	7–19	341	525	184	461	575	114
41183	Sumter clay	7–19	326	537	211	460	525	65
43387	Leaf fine sandy loam	3–7	215	205	0	238	225	0
43392	Tabor fine sandy loam	7–18	400	450	50	370	463	93
43393		19–36	372	290	0	376	288	0
43402	Crockett very fine sandy loam	7–13	379	172	0	390	180	0
44350	Webb fine sandy loam	20–24	390	463	73	420	488	68
Average			267	392	135	301	410	116

evident that the presence of the dicalcium phosphate promoted the conversion of nitrites to nitrates.

In order to test the relative efficiency of various kinds of phosphates, soils were selected in which phosphates increased nitrification, and tests were made

with a number of phosphates in which 0.5 gm. of phosphate and 1 gm. of calcium carbonate were added to 100 gm. of soil. The gains or losses due to the addition of the phosphates are shown in table 3. The different materials are arranged in the approximate order of the average quantity of nitric nitrogen produced. Monopotassium phosphate produced the highest average increase in nitric nitrogen, followed by superphosphate, which produced very nearly the same increase. Monocalcium phosphate and dipotassium phosphate came next. Disodium phosphate, dicalcium phosphate, and

TABLE 3

Gain or loss in nitric nitrogen production due to phosphate addition to soils

SAMPLE NUMBER OF SOIL	MONOPOTASSIUM PHOSPHATE	20 PER CENT SUPERPHOSPHATE	MONOCALCIUM PHOSPHATE	DIPOTASSIUM PHOSPHATE	DISODIUM PHOSPHATE	DICALCIUM PHOSPHATE	TRICALCIUM PHOSPHATE	GROUND ROCK PHOSPHATE	PHOSPHATE ROCK	GROUND TENNESSEE PHOSPHATE	SOFT ROCK PHOSPHATE WITH COLLOIDAL CLAY
Total P_2O_5, *per cent*.....	51.7	20.5	55.7	38.7	26.0	46.9	40.0	24.8	32.5	32.1	20.1
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
39880	327	302	302	254	214	254	289	264	204	160	170
39687	219	178	142	200	189	181	169	123	132	134	125
43402	158	183	96	146	146	121	121	83	83	83	8
39884	125	88	88	100	100	88	100	75	50	50	63
39882	113	75	100	113	88	38	50	63	63	50	13
39686	101	131	90	93	32	100	82	50	31	74	13
43400	100	75	87	75	12	37	50	25	−13	37	37
43397	87	137	50	137	125	75	62	25	25	37	37
43393	75	50	62	62	37	50	50	0	0	12	0
39682	50	37	62	50	50	37	37	25	12	25	37
39879*	50	25	38	38	50	0	0	0	0	0	0
41183	38	75	88	50	63	75	63	25	25	63	50
39689	15	28	65	3	3	−10	−10	−10	−10	15	3
44350	8	46	46	8	8	8	33	8	− 4	11	−4
Average of gains.	105	102	94	95	80	82	85	59	57	54	43

* Sumter clay, 0–7 inches.

tricalcium phosphate produced considerably lower nitrification. Ground rock phosphate (three samples) gave much lower nitrification than the foregoing, and soft phosphate with colloidal clay gave the lowest results. These average results are in the order one would expect from a knowledge of the availability of the phosphoric acid in the phosphates, taking into consideration the quantity of phosphoric acid (P_2O_5) used. The order of effectiveness of the phosphates is not always the same with the individual soils, but bacterial processes cannot be expected to proceed with the exactness of inorganic chemical reactions.

The question arises as to why these soils require phosphates for nitrification. In order to see whether the chemical composition throws any light upon this question, the soils giving the highest response to phosphates were analyzed by methods already described (6). Of the 14 samples examined, 2 are surface soils and 12 are subsurface soils. They are classified as fine sandy loams, very fine sandy loams, clays, and clay loams; the subsurface soils are generally heavier in texture than the surface soils. Of the 14 samples, 7 contain 8 p.p.m. of active phosphoric acid (soluble in 0.2 *N* nitric acid) or less; 4 contain 7 to 34 p.p.m.; and 3 contain between 48 and 69 p.p.m. The acid-soluble lime ranges from 0.18 to 11.80 per cent. The basicity ranges from 0.2 to 25.76 per cent. The pH of the original soils ranges from 4.79 to 8.37. The composition of the soils in which nitrification is increased by the addition of phosphates thus covers a wide range. The prevailing characteristics seem to be low active phosphoric acid and fair to high lime and basicity.

The effect of phosphates on nitrification was studied by Brown and Gawda (2), who reported that rock phosphate and acid phosphates increased the nitrate content and the nitrifying power of Carrington loam. A similar conclusion was reached by Truog et al. (14) also working with Carrington loam. Fraps (5) reported that the addition of phosphate or potash increased nitrification in several soils. Patterson and Scott (12) reported moderate increases in nitrification due to additions of gypsum, superphosphate, and ferric hydroxide. Robinson and Bullis (13) found that monocalcium phosphate had a beneficial effect on nitrification in one acid soil, a depressing effect on three other soils, and caused a slight increase of nitrates in a fifth soil. These investigators reported also that field plot tests did not confirm these results. Mack and Haley (10) reported increases in nitrification caused by monocalcium phosphate. Dean and Smith (3) found that limestone applied with rock phosphate to an acid soil caused a slight increase in nitrification, but rock phosphate alone did not affect nitrification to any appreciable extent. A similar conclusion was reached by Ames and Richmond (1), who made additions of rock phosphate to peat soil and pure quartz sand. Mackenna (11) found that the addition of soluble phosphate increased the rate of ammonification in all of the soils examined, but in many cases it diminished the nitrification, apparently as a result of the disproportionate increase in non-nitrifying organisms. The final result over long periods of time was no increase in the total nitrate formed. Dorsey and Brown (4) found that superphosphate did not increase nitrification in pasture land.

The results reported in this paper are in accord with the conclusions of the aforementioned investigators. Phosphate treatments, especially when calcium carbonate is also present, stimulate very materially the oxidation of ammonia-nitrogen of a large proportion of those soils the low nitrifying capacity of which is not greatly increased by additions of calcium carbonate; whereas in a few of such soils, additions of phosphate have no effect upon nitrification. Thus, the nitrifying capacity of a large percentage of soils

which have a low nitrifying power is increased by additions of calcium carbonate and inoculating liquid, that of many other such soils is increased by phosphates, and that of the remaining few is not highly increased by either treatment.

SUMMARY

Of 171 soil samples, 32 nitrified less than 60 per cent of the ammonia-nitrogen added even though nitrifying organisms and calcium carbonate were added. Addition of phosphates increased nitrifying capacity of most of these soils. Monocalcium phosphate alone increased the nitrification in 4 of 18 such soils. Monocalcium phosphate with calcium carbonate increased the nitrification in 16 of the 18 soils over that with calcium carbonate alone. Dicalcium phosphate increased nitrification in 29 soils which did not completely nitrify when inoculating liquid and calcium carbonate were added. Nitrites were present in some of the cultures, but the number containing nitrites and the quantities present were smaller when phosphates were added than when they were not added.

The average order of effectiveness of phosphates (averages of 14 soils) to promote nitrification, beginning with the most effective, is as follows: monopotassium phosphate, 20 per cent superphosphate, dipotassium phosphate, monocalcium phosphate, tricalcium phosphate, dicalcium phosphate, disodium phosphate, rock phosphates, soft phosphate with colloidal clay. These results are in the order one could expect from the knowledge of the availability of the phosphoric acid in the phosphates.

There are differences in the quantities of the active phosphoric acid, acid-soluble lime, basicity, and pH values of the soils which respond to phosphates, but the prevailing characteristics seem to be low active phosphoric acid and fair to high lime and basicity.

REFERENCES

(1) Ames, J. W., and Richmond, T. E. 1918 Effect of sulfofication and nitrification on rock phosphate. *Soil Sci.* 6: 351–364.

(2) Brown, P. E., and Gawda, R. N. 1924 The effect of certain fertilizers on nitrification. *Jour. Amer. Soc. Agron.* 16: 137–146.

(3) Dean, H. L., and Smith, F. B. 1933 The effect of lime and phosphate on nitrification in an acid soil. *Proc. Iowa Acad. Sci.* 40: 84–85.

(4) Dorsey, H., and Brown, B. A. 1935 Pasture investigations. VI. Seasonal variations in the reaction, nitrates and ammonia of soil from different fertilized permanent pastures. Conn. (Storrs Sta.) Agr. Exp. Sta. Bul. 206.

(5) Fraps, G. S. 1920 Nitrification in Texas soils. Tex. Agr. Exp. Sta. Bul. 259.

(6) Fraps, G. S., and Fudge, J. F. 1937 Chemical composition of soils of Texas. Tex. Agr. Exp. Sta. Bul. 549.

(7) Fraps, G. S., and Sterges, A. J. 1931 Estimation of nitric and nitrous nitrogen in soils. Tex. Agr. Exp. Sta. Bul. 439.

(8) Fraps, G. S., and Sterges, A. J. 1932 Causes of low nitrification capacity of certain soils. *Soil Sci.* 34: 353–363.

(9) FRAPS, G. S., AND STERGES, A. J. 1937 Basicity of some phosphates as related to nitrification. *Jour. Amer. Soc. Agron.* 29: 613–621.

(10) MACK, W. B., AND HALEY, D. E. 1927 The effect of potassium salts on the availability of nitrogen in ammonium sulfate. *Soil Sci.* 25: 333–336.

(11) MACKENNA, J. 1919 Soil biology. (*Rpt. Prog. Agr. India* 1917–18: 101–103). Abs. in *Exp. Sta. Rec.* 41: 720.

(12) PATERSON, J. W., AND SCOTT, P. R. 1913 Influence of certain soil constituents upon nitrification. *Jour. Dept. Agr. Victoria* 10: 393–400.

(13) ROBINSON, R. H., AND BULLIS, D. E. 1922 Acid soil studies: III. The influence of calcium carbonate, calcium oxide, and calcium sulfate on soluble soil nutrients of acid soils. *Soil Sci.* 13: 449–459.

(14) TRUOG, E., HARPER, H. J., et al. 1925 Fertilizer experiments: Methods of application and effect on germination, early growth, hardiness, root growth, lodging, maturity, quality and yield. Univ. Wis. Res. Bul. 65.

THE STIMULATING EFFECTS OF SILICATES ON PLANT YIELDS IN RELATION TO ANION DISPLACEMENT[1]

S. J. TOTH

New Jersey Agricultural Experiment Station

Received for publication July 16, 1938

The release of fixed phosphates from soils by the addition of silicates has been attributed by investigators to a resulting anion exchange reaction (15, 21). In a previous paper (19), the mechanism of displacement of silicate ions from soil colloids by means of phosphate ions was elucidated. The displacement of native phosphates from soil colloids, however, was not observed when the colloids were suspended in acid solutions of sodium silicate. This was believed to be due to the decreased activity of silicate ions under acid conditions.

The present investigation is, in certain respects, a continuation of the previous one, that is, the displacement of adsorbed phosphate ions from soils by means of silicate ions under varying pH values was studied, as well as the rôle of silicate ions in increasing available soil phosphates. The effects of silicate additions to soils on plant yields and composition were also investigated. The displacement experiments were conducted with Colts Neck loam rather than with colloids, since the soil was also used for the growing of crops in greenhouse experiments.

EXPERIMENTAL METHODS

Colts Neck loam was phosphated by suspending 750 gm. of the H-soil in 7,500 cc. of 0.046 *N* H_3PO_4 adjusted with dilute NH_4OH to give to the suspension, after adsorption, a pH between 4.0 and 4.2. As the adsorption of the ammonium ion is small at these pH values, the soil is considered, in the later discussions, to be essentially H-phosphated. The soil, after adsorption of the phosphate ions, was transferred to a Büchner funnel and drained of excess liquid by suction. The soil in the funnel was washed twice with 500-cc. portions of distilled water, air-dried, and passed through a 1-mm. sieve. The value for the quantity of phosphate ion retained by the soil was obtained by determining the total phosphoric acid content of the original soil and that of the phosphated soil.

The procedure in the phosphate-ion displacement experiments was to suspend a definite weight of the phosphated soil in 100 cc. of solution con-

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

taining increments of the displacing anions. The specific effect of different cations on the dissociation of the adsorbed ion and on the dispersion of the soil particles was minimized by using all of the displacing anions as the sodium salt. The averages of triplicate systems are reported in the tables 1, 2, 3, and 4.

The displacing experiments were conducted in systems that were allowed to stand for two different time intervals. The two systems are recorded in the tables as 2- and 14-day intervals. At the completion of the time intervals, an aliquot of the supernatant liquid was withdrawn for determinations of pH and of displaced and adsorbed ions. The aliquot from the hydroxide and silicate systems at high pH values contained considerable quantities of organic matter. The organic matter was removed by oxidation with H_2O_2 prior to stabilization of the aliquots to remove silica. Soil dispersion occurred in some of the lower ion concentrations, but in these instances a clear supernatant liquid was obtained by flocculating the dispersed particles with 20 m.e. of sodium chloride.

Crops grown in the pots were harvested at or near maturity, and the recorded dry weights represent the average yields of duplicate pots. The plant samples were analyzed in duplicate for nitrogen, phosphorus, silica, calcium, and magnesium by the methods outlined by the A. O. A. C. The analyses are reported on samples that were dried to constant weight at 80°C.

The available phosphate content of the pot soils was determined in duplicate by the method of Truog (20). The electrodiffusible phosphorus content of certain pot soils was obtained by electrodialyzing 20 gm. of the soil in a modified Mattson cell at 0.20 ampere. An aliquot of the anolyte was analyzed for the phosphate content by the method of Parker and Fudge (12).

DISPLACEMENT OF ADSORBED PHOSPHATE IONS FROM COLTS NECK LOAM BY HYDROXIDE AND SILICATE IONS

Certain important points with reference to the conditions under which the phosphate ions were fixed by the soil must be remembered in evaluating the data to be presented. In order to obtain conditions similar to those encountered in acid field soils, the fixation of the phosphate ions was allowed to result at low pH values (4.0–4.2). The fixed phosphates, therefore, were largely in the unavailable forms. In a previous paper (19), it was shown that the colloid extracted from this soil fixed soluble phosphate ions by the following series of reactions: fractions of the adsorbed phosphate displaced silicate ions from the lattice of the clay; another fraction reacted with the free iron oxides; and a fraction displaced hydroxyl groups from uncombined basoid.

The displacement of the adsorbed phosphate ions by other anions resolves itself into a study of the release of the ions fixed by the various mechanisms described above. Evidence has been presented by Scarseth (15) which seems to indicate that the fixation and release of the phosphate ions fixed through the uncombined basoid fraction of colloids is rapid. Since the

Colts Neck soil contains approximately 19 per cent of free iron oxides, it seems that the fixation of phosphate by this soil at low pH values is principally due to the first two mechanisms listed above.

The data for the adsorbed phosphorus content of the phosphated soil are given below:

Concentration of P_2O_5 per gram of soil after adsorption	=	14.72 mgm.
Initial concentration of P_2O_5 per gram of soil	=	5.87 mgm.
Adsorbed phosphate content per gram of soil	=	8.85 mgm.

The quantity of soil used in the displacement studies contained 1.59 m.e. of adsorbed P_2O_5. Approximately 0.6 per cent of the adsorbed phosphate content of this soil was soluble in distilled water. The anions employed as displacing ions were the hydroxide, silicate, silicate neutralized with dilute

TABLE 1

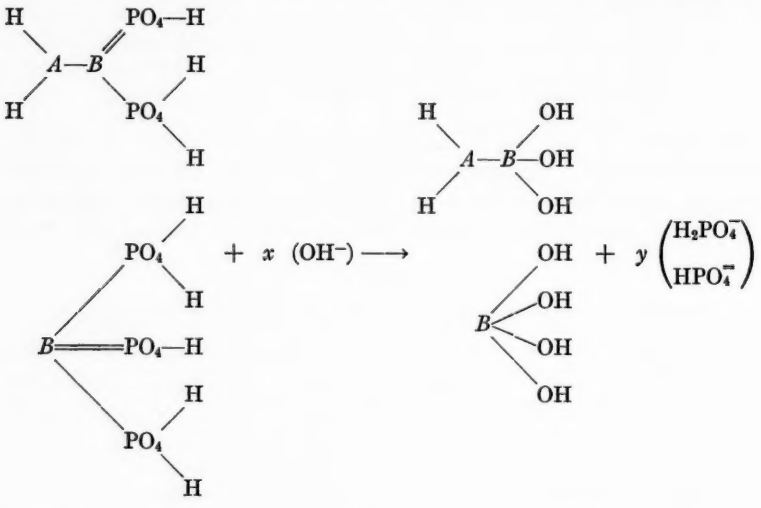
Displacement of adsorbed phosphate ions from Colts Neck H-loam by hydroxyl ions

CONCENTRATION OF NaOH	pH OF SUPERNATANT LIQUID		P_2O_5 RELEASED		REPLACEMENT IN 14-DAY SYSTEM
	2 days	14 days	2 days	14 days	
m.e./100 cc.			*m.e.*	*m.e.*	*per cent*
0.1	5.75	5.75	0.11	0.15	9.4
0.5	7.25	6.22	0.25	0.48	30.1
1.0	9.10	6.85	0.42	0.53	33.3
2.0	11.25	8.75	0.60	0.84	52.8
2.5	11.35	10.00	0.83	1.01	63.5
3.0	11.70	10.39	0.97	1.09	68.5
5.0	12.30	12.30	1.24	1.31	82.3
10.0	12.50	12.50	1.48	1.59	100.0

HCl to pH 7.0, and the sulfate. The data obtained with these anions are presented in tables 1, 2, 3, and 4.

From the data in table 1, it can be noted that a complete release of the adsorbed phosphate ions was attained under conditions of high hydroxyl-ion concentrations, when sufficient time was allowed for the reaction to proceed to completion. Apparently the reactions between the hydroxyl and the fixed phosphate ions are not instantaneous, since the mobilization of the latter ions are greater in the 14-day than the 2-day systems. Approximately one-third of the adsorbed ions are displaced by the hydroxyl ions at pH values less than 7.0. The displacement of the residual adsorbed phosphate ions resulted only under conditions of high hydroxyl-ion concentration. This is shown by the fact that only 82.3 per cent of the total was released at pH 12.3. This behavior points to a difference in the strength of the linkages between the adsorbed phosphate ions and the soil complex. Degradation of the phosphated soil complex resulted at pH values greater than 7.0, and manifestations of this were indicated by the increased solubility of humus

and silica. These results are in line with the findings of Ravikovitch (13) and Demolon (2, 3). The mechanism of the displacement of the adsorbed phosphate ions by the hydroxyl ions may be represented diagrammatically as follows where *A* and *B* have the same significance as outlined previously (19):



(I) (II)

The adsorbed phosphate ions in complex I are represented as integral parts of a new phosphated ionogen. This is in line with the fact that the adsorption of phosphate ions by soil colloids results in an increase in cation exchange capacity and a decrease in ultimate pH (19).

There is only a slight possibility of the release of adsorbed phosphate ions through a mechanism of anion exchange which is similar in nature to an equivalent cation exchange reaction. This is due to the fact that the phosphate ions associate strongly with the complex (10) through the uncombined basoid residues. The linkage between the complex and the strongly associating anions must be strong, for the easily dissociated Cl^-, NO_3^-, and $SO_4^=$ ions are easily displaced below pH 7.0, whereas this does not occur with the phosphate ions. The displacement of phosphate ions by anions of sodium silicate solutions under alkaline conditions is not simple, as is the case with the weakly associating ions. The replacement is the result of degradation of phosphated complex under conditions of high hydroxyl-ion concentration with the release of silica, humus, and phosphorus. A reaction of this nature probably should be designated as an acidoid displacement to distinguish it from the exchange of the easily dissociated anions. There is evidence in the literature that indicates that the displacement of phosphate ions by organic anions such as the citrates and tartrates (2) is a reaction of this type.

The displacing power of the hydroxyl ion reported in table 1 was compared with the displacing action of silicate solutions. For this purpose two series of sodium silicate solutions were used: one, the usual alkaline solution, and the other, a dilute solution brought to pH 7.0 with HCl. In the preparation of the latter solution, approximately 0.1 *N* sodium silicate was acidified to pH 7.0 and aged for 2 weeks. The solution was stable, as shown by the absence of free silicic acid flocs. The neutralized sodium silicate solution when used in the displacing studies contained sufficient sodium hydroxide, which was added simultaneously with the silicate solution, to insure a final pH of approximately 7.0 in the supernatant liquid of the system. The quantity of sodium hydroxide added to the system was determined from the data reported in table 1.

TABLE 2

Displacement of adsorbed phosphate ions from Colts Neck H-loam by ions of sodium silicate solutions

CONCENTRATION OF SiO_2 AS Na_2SiO_3	pH OF SUPERNATANT LIQUID (UNNEUTRALIZED Na_2SiO_3)		pH OF SUPERNATANT LIQUID (NEUTRALIZED Na_2SiO_3)		P_2O_5 RELEASED (UNNEUTRALIZED Na_2SiO_3)		P_2O_5 RELEASED (NEUTRALIZED Na_2SiO_3)		REPLACEMENT IN 14-DAY SYSTEM (UNNEUTRALIZED Na_2SiO_3)	REPLACEMENT IN 14-DAY SYSTEM (NEUTRALIZED Na_2SiO_3)
	2 days	14 days	2 days	14 days	2 days	14 days	2 days	14 days		
m.e./100 cc.					*m.e.*	*m.e.*	*m.e.*	*m.e.*	*per cent*	*per cent*
0.5	.....	.....	6.96	7.10	....	....	0.23	0.24	....	15.0
1.0	.....	.....	6.96	7.05	....	....	0.22	0.30	....	18.8
2.5	9.05	6.90	6.85	7.00	0.51	0.52	0.20	0.36	32.7	22.6
5.0	10.10	8.75	6.75	6.95	0.92	1.09	0.17	0.40	68.5	25.1
7.5	.....	.....	6.70	7.00	....	....	0.17	0.38	....	23.8
10.0	11.17	11.00	6.85	6.98	1.23	1.48	0.13	0.35	93.0	22.0
13.0	.....	.....	6.85	6.86	....	....	0.13	0.38	....	23.8
15.0	12.20	12.15	....	....	1.31	1.52	....	....	95.5	....
20.0	12.35	12.30	....	....	1.34	1.51	....	....	95.0	....
25.0	12.40	12.40	....	....	1.34	1.52	....	....	95.5	....

The data obtained with the two silicate ion series are presented in table 2. Progressive increments of adsorbed phosphate ions were displaced by increasing concentrations of SiO_2 in the alkaline silicate systems in a manner analogous to that found with the hydroxide system. It is to be noted from table 2, that with the 2-day system of unneutralized silicate solution, the displacements of adsorbed phosphate ions are greater than those in the hydroxyl-ion system when comparisons are made at approximately equivalent pH values. This behavior would tend to indicate that the displacement of the adsorbed phosphate ions in these systems is the result of the combined effects of silicate and hydroxyl ions. The data obtained with the 14-day systems, however, do not confirm the previous belief, and it seems that the displacement of adsorbed phosphate by ions of sodium silicate solutions is governed perhaps only by the pH of the systems. A further confirmation of this may be noted from the data obtained with the neutral sodium silicate

system. The smaller quantity of the displaced phosphate ions contained in this system when compared with the unneutralized silicate and hydroxyl ion systems, at equivalent pH values, cannot be explained at present, but this point is being investigated. The results with the neutralized silicate solution are not in line with the findings of other investigators (14, 15). This fact may be attributed to the manner in which the various experiments were performed. In the systems investigated by Scarseth (15) a competition existed between the phosphate and silicate ions in solution to associate with the complex. It is to be expected that under these conditions, as the concentration of one of the competing ions increased the adsorption of the other decreased. The conditions involved in systems investigated in this paper are not similar to those of the aforementioned authors in that a competition of this nature is not to be found in the systems studied.

The displacement data obtained with the sulfate ion as the displacing anion are reported in table 3. In many respects the data for this system are

TABLE 3

Displacement of adsorbed phosphate ions from Colts Neck H-loam by sulfate ions

CONCENTRATION OF Na_2SO_4	pH OF SUPERNATANT LIQUID		P_2O_5 RELEASED		REPLACEMENT IN 14-DAY SYSTEM	CONCENTRATION OF $SO_4^=$ AFTER ADSORPTION	
	2 days	14 days	2 days	14 days		2 days	14 days
m.e./100 cc.			*m.e.*	*m.e.*	*per cent*	*m.e.*	*m.e.*
2.5	6.60	7.00	0.14	0.24	15.0	3.27	2.86
5.0	6.60	6.84	0.13	0.25	16.7	5.87	5.46
10.0	6.60	6.72	0.13	0.24	15.0	10.76	10.45
15.0	6.52	6.84	0.11	0.24	15.0	15.80	15.28
20.0	6.40	6.84	0.10	0.22	13.8	22.14	20.25
25.0	6.60	6.92	0.10	0.20	12.1	27.55	25.45

similar to those obtained with the neutralized silicate system. As in the latter system, the increasing concentration of the displacing anion did not produce increasing replacement of the adsorbed phosphate ions. A greater displacement of the retained ion from the soil was noted, however, with the highest concentration of SiO_2 (about 13 m.e. of SiO_2). Approximately 23.8 per cent of the phosphate ions were released in the latter system, whereas only 12.1 per cent were released when the concentration of the sulfate ion amounted to 25 m.e., or nearly double the highest silicate concentration.

The anions studied can be classified into two groups with respect to the mobilization of fixed phosphate ions. The hydroxide and the alkaline silicate constitute the ions of the first group, and the neutralized silicate and the sulfate, the anions of the second group. The associating tendency of the hydroxyl ion with the soil complex has long been recognized by investigators. The weaker displacing power of the sulfate ions is due to the fact that this anion cannot disrupt the micelle nucleus at pH values near neutrality.

Although the data presented in table 2 indicate that the silicate ions do not participate to any marked extent in the acidoid displacement at alkaline pH values, this anion is adsorbed by the soil during the displacement. The data obtained with the two silicate systems with reference to this point are presented in table 4. The adsorption of silicate ions may be due to the formation of silicate combinations with the soil basoids (Fe and Al) and seems to increase, as would be expected, with increasing silicate-ion concentration and pH.

The anion displacement experiments have been conducted with essentially Na-saturated soils (the result of the exchange between H^+ ion in the complex,

TABLE 4

Adsorption of silicate ions by Colts Neck H-loam

CONCENTRATION of SiO_2 AS Na_2SiO_3	pH OF SUPERNATANT LIQUID (UNNEUTRALIZED Na_2SiO_3)		SiO_2 ADSORBED (UNNEUTRALIZED Na_2SiO_3)		pH OF SUPERNATANT LIQUID (NEUTRALIZED Na_2SiO_3)		SiO_2 ADSORBED (NEUTRALIZED Na_2SiO_3)	
	2 days	14 days	2 days	14 days	2 days	14 days	2 days	14 days
m.e./100 cc.			*m.e.*	*m.e.*			*m.e.*	*m.e.*
0.50	.....	.....	....	....	6.96	7.10	....	....
1.00	.....	.....	....	....	6.96	7.05	....	....
2.50	9.05	6.90	1.10	1.70	6.85	7.00	0.12	....
5.00	10.10	8.75	2.81	3.43	6.75	6.95	0.37	0.26
7.50	.....	.....	.....	.....	6.70	7.00	0.49	0.62
10.00	11.17	11.00	4.18	4.73	6.85	6.98	0.56	....
13.00	.....	.....	.....	.....	6.85	6.86	3.48	4.10
15.00	12.20	12.15	5.14	5.57	....	....	....	....
20.00	12.35	12.30	6.02	6.45	....	....	....	....
25.00	12.40	12.40	7.55	7.50	....	....	....	....

and Na^+ ions from the salts used). The general trends obtained will still hold even under conditions of divalent (Ca^{++}) cation saturation, though variations will exist because of the possibility of insoluble salt precipitation reactions.

GREENHOUSE EXPERIMENTS WITH SILICATES AND LIME

The soil used in the pot experiments with silicates, as stated previously, was the A horizon of virgin Colts Neck loam. The partial chemical composition and certain physicochemical properties of this soil are presented below:

SiO_2/R_2O_3	= 4.36
P_2O_5	= 0.59 per cent
CaO	= 0.24 per cent
MgO	= 0.75 per cent
C	= 1.84 per cent
Field pH	= 4.2
Cation exchange capacity	= 12.1 m.e. per 100 gm.

Calcium and magnesium silicates were used in these pot experiments because the introduction of these materials into the soil created a more favorable medium for plant growth than did the alkali silicates. For comparison with the silicates, a hydrated lime series was included in the experiment. The application of the silicates was based on the lime or magnesia content of these salts and was made equivalent to the lime content of the hydrated lime applications. The nitrogen, potash, and phosphorus sources were commercial products.

On the silicate and hydrated lime series, three phosphorus carriers were compared; namely, raw rock phosphate, superphosphate, and diammonium phosphate. The initial phosphate application was made on the basis of the phosphoric acid content of 250, 500, and 1000 pounds of 16 per cent superphosphate. Later the applications were increased to 750, 1500, and 3000 pounds of 16 per cent superphosphate per acre.

The lime, phosphate, and silicate applications to the pots were thoroughly mixed throughout the soil. The nitrogen and potash sources were applied in solution to the surface of the soils. After the addition of the different fertilizers to the soils, the pots were made up to the optimum moisture content and were kept in this state for 1 week to allow fixation or displacement reactions to attain equilibrium. At the end of this period, several small borings of soil were withdrawn from duplicate pots, mixed, and air dried for available phosphate determinations. A uniform number of seedlings was allowed to grow to maturity or almost to maturity in the pots. Soil samples were withdrawn again from the pots after the crops were harvested.

EFFECTS OF PHOSPHATE APPLICATIONS IN CONJUNCTION WITH HYDRATED LIME OR CALCIUM AND MAGNESIUM SILICATES UPON YIELDS OF SOYBEANS

The lime series received 17.5 gm. of the hydroxide, and the two series designated as calcium and magnesium silicates, 19.5 and 16.9 gm. of the former and the latter salts. Approximately 10 gm. of SiO_2 or 1.1 tons of silica per acre was added in the application of silicates. Sufficient nitrogen ($NaNO_3$) and potash (KCl) were added to the pots to satisfy the nutritional needs of the soybeans.

The dry weight yields of soybeans (Dunnfield variety) from the various series are presented in table 5. It is apparent from these data that the addition of basic materials to the soil, either in the form of hydrated lime or in the form of silicates, increased the production of dry matter. The applications of phosphorus simultaneously with lime generally affected the yields in proportion to the quantities of phosphate applied. This tendency was observed also with the rock phosphate on the magnesium silicate series. The yields obtained from the calcium silicate series seem to be independent of the amount and nature of the phosphate application. The yields obtained from the pots receiving no phosphate applications are exceedingly interesting.

The greatest yields were obtained with the calcium silicate series, followed by the hydrated lime series, then the magnesium silicate, and finally the check. These results indicate a definite stimulation of dry matter production by the application of calcium silicate and are in line with the findings of other investigators (4, 7, 8, 9).

EFFECTS OF ADDITIONAL APPLICATIONS OF SILICATES AND LIME WITHOUT PHOSPHATES

After the removal of the soybean crop from the pots, the soil in the limed series received an additional 10 gm. of hydrated lime. The corresponding calcium and magnesium silicate applications were 14.45 and 12.5 gm. of the

TABLE 5

Dry weight yields of soybeans

PHOSPHORUS FORM	P_2O_5 PER ACRE	YIELDS OF SOYBEANS			
		No special treatment	Hydrated lime series	Calcium silicate series	Magnesium silicate series
	lbs.	*gm.*	*gm.*	*gm.*	*gm.*
None	...	5.80	14.00	18.05	7.85
Raw rock phosphate	40	7.30	14.45	17.75	9.75
	80	7.95	15.80	17.75	11.85
	120	8.37	18.25	17.35	13.10
Superphosphate	40	6.90	19.50	17.95	11.15
	80	7.80	20.00	16.25	13.50
	120	8.70	20.00	17.80	12.65
Diammonium phosphate	40	7.15	19.75	16.55	10.25
	80	7.85	20.50	16.85	12.95
	120	8.35	25.00	17.95	12.80
Soil pH		4.60	5.30	5.00	4.80

former and latter salt. The silicate treatments were equivalent to 0.7 ton of SiO_2 per acre. One gram of nitrate of soda and 1 gm. of muriate of potash were added to the pots, which were then seeded to rape.

A very high application of silicates, equivalent to 5 tons of SiO_2 per acre, was added to the pots after the harvesting of the rape crop. This high application was necessary because only slight effects of the silicate treatment had been observed in the composition of the plant material grown on the silicated soils. The large application of silicates necessitated a correspondingly large application of hydrated lime. As the result of this treatment, the pH values on this section are slightly above 7.0. The small yields of rape from the magnesium silicate section were thought to be due to a poor balance of exchange CaO to MgO. To remedy this condition, 5.0 gm. of

calcium sulfate were added to the pots before seeding to barley. The series designated in table 6 as "precipitated calcium silicate," was the original unlimed series which received 20 gm. of precipitated calcium silicate. This special treatment was included to observe whether or not the precipitated material would induce more marked effects than the salt prepared by fusing SiO_2 with CaO. The dry weight yields of rape and barley are presented in table 6.

The beneficial influences of the initial phosphate application are still to be noted in the yields of rape from the limed and silicated series. The yields from these special treatments seem to depend upon the initial application of

TABLE 6

Dry weight yields of rape and barley

PHOSPHORUS FORM	P_2O_5 PER ACRE	YIELDS OF RAPE			YIELDS OF BARLEY			
		Hydrated lime series	Calcium silicate series	Magnesium silicate series	Precipitated calcium silicate series	Hydrated lime series	Calcium silicate series	Magnesium silicate series
	lbs.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
None	0	3.75	5.50	0.72	2.80	6.35	18.30	13.20
Raw rock phosphate	40	2.00	7.75	1.92	4.60	7.60	17.75	12.75
	80	6.00	8.25	1.20	3.80	8.65	19.00	13.35
	120	10.25	11.25	5.05	5.35	8.60	18.75	13.75
Superphosphate	40	5.25	8.00	2.50	4.25	9.10	14.45	13.75
	80	9.75	10.25	1.85	3.40	10.65	18.00	12.90
	120	12.50	11.87	4.45	7.25	10.05	17.25	13.60
Diammonium phosphate	40	6.75	4.25	1.11	5.25	11.65	17.40	12.00
	80	9.75	8.00	1.03	4.50	10.50	19.05	12.55
	120	11.75	12.75	2.12	4.80	12.00	19.70	14.25
Soil pH		5.80	5.20	4.80	4.60	7.05	6.30	5.40

phosphates to the soils. These results differ from the yields of soybeans in that with this crop the yields from the calcium silicated soil also seemed to be dependent on the phosphate applications. Very slight differences exist between the yields of rape from the limed and from the calcium silicated series. The yields from these series, however, are greater than the yields from the magnesium silicate section. As has been suggested, the small yields from this section are due to an unfavorable ratio of exchange CaO to MgO in the complex brought about by the addition of magnesium silicate and the removal of exchange calcium by the soybean crop. These points are verified by the fact that where superphosphate had been applied at a rate corresponding to 1000 pounds per acre, the yields are greater than from

the corresponding diammonium phosphate application. The yields from the unlimed series are not reported in table 6 because of the small production of dry matter.

Table 6 presents the yields of barley from the various special series. The high application of silicates markedly increased the yield of barley, as indicated by the data in the table 6. The increased yields of barley from the magnesium silicate sections are due not only to the production of a more favorable exchange CaO/MgO ratio in the soil brought about by the addition of 5 gm. of $CaSO_4$ per pot, but also to the effect of the silicates. The yield responses of barley to the silicate applications are in line with the findings of Schollenberger (16), Fisher (4), Gile and Smith (6), and others. The relative yields from the check pots of the special treatments, assuming that the yield from the limed soils are normal, are approximately 2.9 times as great and 2.0 times as great with the calcium and magnesium silicate treatments. The question might be raised as to whether or not the yield of barley from the limed soils should be considered normal, inasmuch as the soil in this series had a pH value slightly above 7.0. Observations on the barley plants during the growing season failed to indicate any external differences between the plants on this series and those on the others. The effects of the initial application of phosphates are not noticeable in the yields of barley from the various series reported in table 6.

RESIDUAL EFFECTS OF SILICATE APPLICATIONS ON YIELDS OF SUDAN GRASS

The pot soils had received approximately 6.8 tons of silica over a period of three growing seasons. To determine whether or not the additions of silica exerted any residual effects upon the availability of phosphates, the soil was again treated with phosphate. The application of phosphate salts was increased to 120, 240, and 480 pounds of P_2O_5 on the acre basis. Proper adjustments were made to all pots, with respect to nitrogen, to equalize the effect of the nitrogen content in the diammonium phosphate. This was done by using ammonium sulfate.

The dry weight yields of sudan grass from the various sections are presented in table 7. Plate 1 illustrates the effects of lime and silicates on the yield of sudan grass. It is of interest to compare the yields of the check pots of the three series; again the calcium silicated soils produced larger yields than did the other treatments. The two silicated series produced more dry matter than did the limed series. These results are similar to the yields obtained with barley on the calcium silicated section. As with the latter series, the yields from these two sections, though showing increases over the checks, seem to be independent of the phosphate application.

The specific behavior of the three phosphate salts on the limed soil is very apparent from the data in table 7. The smaller yields from the rock phosphate section of this series may be accounted for by the decreased availability

calcium sulfate were added to the pots before seeding to barley. The series designated in table 6 as "precipitated calcium silicate," was the original unlimed series which received 20 gm. of precipitated calcium silicate. This special treatment was included to observe whether or not the precipitated material would induce more marked effects than the salt prepared by fusing SiO_2 with CaO. The dry weight yields of rape and barley are presented in table 6.

The beneficial influences of the initial phosphate application are still to be noted in the yields of rape from the limed and silicated series. The yields from these special treatments seem to depend upon the initial application of

TABLE 6

Dry weight yields of rape and barley

PHOSPHORUS FORM	P_2O_5 PER ACRE	YIELDS OF RAPE			YIELDS OF BARLEY			
		Hydrated lime series	Calcium silicate series	Magnesium silicate series	Precipitated calcium silicate series	Hydrated lime series	Calcium silicate series	Magnesium silicate series
	lbs.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
None	0	3.75	5.50	0.72	2.80	6.35	18.30	13.20
Raw rock phosphate	40	2.00	7.75	1.92	4.60	7.60	17.75	12.75
	80	6.00	8.25	1.20	3.80	8.65	19.00	13.35
	120	10.25	11.25	5.05	5.35	8.60	18.75	13.75
Superphosphate	40	5.25	8.00	2.50	4.25	9.10	14.45	13.75
	80	9.75	10.25	1.85	3.40	10.65	18.00	12.90
	120	12.50	11.87	4.45	7.25	10.05	17.25	13.60
Diammonium phosphate	40	6.75	4.25	1.11	5.25	11.65	17.40	12.00
	80	9.75	8.00	1.03	4.50	10.50	19.05	12.55
	120	11.75	12.75	2.12	4.80	12.00	19.70	14.25
Soil pH		5.80	5.20	4.80	4.60	7.05	6.30	5.40

phosphates to the soils. These results differ from the yields of soybeans in that with this crop the yields from the calcium silicated soil also seemed to be dependent on the phosphate applications. Very slight differences exist between the yields of rape from the limed and from the calcium silicated series. The yields from these series, however, are greater than the yields from the magnesium silicate section. As has been suggested, the small yields from this section are due to an unfavorable ratio of exchange CaO to MgO in the complex brought about by the addition of magnesium silicate and the removal of exchange calcium by the soybean crop. These points are verified by the fact that where superphosphate had been applied at a rate corresponding to 1000 pounds per acre, the yields are greater than from

the corresponding diammonium phosphate application. The yields from the unlimed series are not reported in table 6 because of the small production of dry matter.

Table 6 presents the yields of barley from the various special series. The high application of silicates markedly increased the yield of barley, as indicated by the data in the table 6. The increased yields of barley from the magnesium silicate sections are due not only to the production of a more favorable exchange CaO/MgO ratio in the soil brought about by the addition of 5 gm. of $CaSO_4$ per pot, but also to the effect of the silicates. The yield responses of barley to the silicate applications are in line with the findings of Schollenberger (16), Fisher (4), Gile and Smith (6), and others. The relative yields from the check pots of the special treatments, assuming that the yield from the limed soils are normal, are approximately 2.9 times as great and 2.0 times as great with the calcium and magnesium silicate treatments. The question might be raised as to whether or not the yield of barley from the limed soils should be considered normal, inasmuch as the soil in this series had a pH value slightly above 7.0. Observations on the barley plants during the growing season failed to indicate any external differences between the plants on this series and those on the others. The effects of the initial application of phosphates are not noticeable in the yields of barley from the various series reported in table 6.

RESIDUAL EFFECTS OF SILICATE APPLICATIONS ON YIELDS OF SUDAN GRASS

The pot soils had received approximately 6.8 tons of silica over a period of three growing seasons. To determine whether or not the additions of silica exerted any residual effects upon the availability of phosphates, the soil was again treated with phosphate. The application of phosphate salts was increased to 120, 240, and 480 pounds of P_2O_5 on the acre basis. Proper adjustments were made to all pots, with respect to nitrogen, to equalize the effect of the nitrogen content in the diammonium phosphate. This was done by using ammonium sulfate.

The dry weight yields of sudan grass from the various sections are presented in table 7. Plate 1 illustrates the effects of lime and silicates on the yield of sudan grass. It is of interest to compare the yields of the check pots of the three series; again the calcium silicated soils produced larger yields than did the other treatments. The two silicated series produced more dry matter than did the limed series. These results are similar to the yields obtained with barley on the calcium silicated section. As with the latter series, the yields from these two sections, though showing increases over the checks, seem to be independent of the phosphate application.

The specific behavior of the three phosphate salts on the limed soil is very apparent from the data in table 7. The smaller yields from the rock phosphate section of this series may be accounted for by the decreased availability

of this material under conditions of high liming. This effect has been observed by many investigators (5, 22). The diammonium phosphate series produced the greatest yields in the hydrated lime series. It is only this special treatment of the three series that shows a definite increase in yields with progressive increments of applied phosphorus.

SILICA AND PHOSPHORUS CONTENTS OF VARIOUS CROPS AS AFFECTED BY FERTILIZATION WITH PHOSPHATES AND SILICATES

Many investigators (7, 8, 9, 16, 21) have pointed out that plants grown on silicated soils differ in chemical composition from those grown on normal soils. The important differences are changes in the silica and phosphorus

TABLE 7

Dry weight yields of sudan grass

PHOSPHORUS FORM	P_2O_5 PER ACRE	YIELDS OF SUDAN GRASS		
		Hydrated lime series	Calcium silicate series	Magnesium silicate series
	lbs.	*gm.*	*gm.*	*gm.*
None	0	3.60	29.00	21.45
Raw rock phosphate	120	2.55	32.75	29.50
	240	4.45	30.00	30.00
	480	6.00	35.50	32.00
Superphosphate	120	6.95	35.50	30.70
	240	18.00	35.00	31.75
	480	17.50	37.00	31.00
Diammonium phosphate	120	18.75	33.50	31.00
	240	25.45	35.50	32.75
	480	28.25	35.50	30.50
Soil pH		6.80	6.10	5.10

content. Table 8 shows the average values of the phosphorus and silica contents of the crops with the different sources and rates of phosphorus applications. These values are compared, therefore, only with respect to the special treatments.

Several outstanding facts may be noted from the data in table 8. The rape and sudan grass show an increased phosphate content when grown on calcium silicated soils. This effect is not noticeable with soybeans and barley. If the comparisons are made on the basis of the total amount of phosphorus recovered in the crop, the recovery with rape, barley, and sudan grass crops is greater on the calcium silicated soils than on the limed.

The absorption of increased amounts of phosphate is generally associated with a higher absorption of silica. This fact is in line with the finding of

many investigators (7, 8, 9, 16). The data in table 8 indicate that plants vary greatly in their ability to absorb silica. Schollenberger (16) has pointed out that the greatest absorption of silica was noted with oats, the smallest with buckwheat. Of the four crops grown during the progress of the experiment, soybeans and rape (0.08–1.0 per cent SiO_2) can be grouped as low-silica plants, and barley and sudan grass (0.6–4.0 per cent SiO_2) as high-silica plants. This grouping recognizes the fact that the grasses generally contain more silica than do other species of plants (16), since with these plants the absorption and deposition of silica in certain portions of the plant acts as a mechanical strengthening agent. Only a slight increase in plant silica is observed with

TABLE 8

Silica and phosphorus contents of crops in relation to special treatments

SPECIAL TREATMENT	SOYBEANS		RAPE		BARLEY		SUDAN GRASS	
	SiO_2	P_2O_5	SiO_2	P_2O_5	SiO_2	P_2O_5	SiO_2	P_2O_5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	0.885	0.384	.....	.....	.....	.....	.....	.....
Hydrated lime	0.755	0.475	0.085	0.485	0.661	0.254	0.611	0.287
Calcium silicate	1.092	0.432	0.234	0.588	3.200	0.239	4.330	0.322
Calcium silicate (ppt.)	.....	.....	.....	.....	3.630	0.366	.....	.....
Magnesium silicate	1.253	0.500	0.322	0.662	4.110	0.343	3.980	0.278

TABLE 9

Partial chemical composition of crops in relation to lime and silicate fertilization

SPECIAL TREATMENT	SOYBEANS			RAPE			BARLEY			SUDAN GRASS		
	N	CaO	MgO	N	CaO	MgO	N	CaO	MgO	N	CaO	MgO
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Hydrated lime	3.22	1.76	0.75	4.77	4.98	0.62	3.07	1.08	0.63	2.21	2.10	0.48
Calcium silicate	3.34	1.42	0.76	4.55	4.09	0.60	1.88	0.56	0.50	1.75	1.00	0.75
Magnesium silicate	3.59	0.50	1.06	4.95	1.68	2.50	2.29	0.28	0.89	1.75	0.33	1.04

the soybean crop grown on the silicated soils when compared with the lime section, but with the other crops increases corresponding to 3–800 per cent were found.

The increased absorption of phosphates by plants grown on silicated soils may be the result of some mechanism functioning either in the soil or within the plant (1, 11, 18). The available phosphate data of the soil in relation to yield and phosphate content of the crops will be discussed later.

The calcium, magnesium, and nitrogen contents of the high phosphorus treatment of all the crops are presented in table 9. The recorded values represent the average content of these constituents in the plants grown on the soils receiving the highest rates of application of the different forms of

phosphates. The total nitrogen content of soybeans and rape grown on the silicated soils does not differ markedly from the total nitrogen content of the plants grown on the limed section. On the other hand, the nitrogen content of barley and sudan grass plants from the silicated soil is lower than that of the plants from the limed soil. This is, of course, due to the increased yields on the former section. Although data for the calcium and magnesia contents of soybeans grown on the unlimed section are not reported in table 9, the average percentages of these constituents are 0.62 and 0.80 per cent respectively. The effects of the hydrated lime applications to the soil are reflected in the increased calcium content of the plants. The absorption of calcium

TABLE 10

Available phosphate content, in pounds per acre, of soils in relation to lime and silicate treatments

SPECIAL TREATMENT	CHECK	RAW ROCK PHOSPHATE			SUPERPHOSPHATE			DIAMMONIUM PHOSPHATE		
		P*	2P	4P	P	2P	4P	P	2P	4P
Soybeans										
None	22.2	23.8	31.9	39.6	24.1	27.0	30.8	20.4	25.2	27.7
Hydrated lime	17.0	25.8	29.7	45.2	21.7	28.1	29.0	19.4	30.4	34.6
Calcium silicate	17.1	30.8	39.1	47.9	22.9	27.0	38.1	19.5	27.3	31.0
Magnesium silicate	22.8	30.6	35.5	45.5	27.3	32.4	36.7	27.7	26.8	44.0
Rape										
Hydrated lime	20.0	23.5	34.9	51.1	30.9	35.5	47.5	25.9	33.5	36.7
Calcium silicate	20.8	23.2	27.3	42.5	16.3	20.0	29.0	17.7	21.1	25.9
Magnesium silicate	13.6	17.6	23.3	30.0	19.7	19.2	22.5	21.3	22.3	22.7
Barley										
Hydrated lime	19.9	20.3	32.5	44.6	26.0	29.7	29.0	26.7	26.6	29.5
Calcium silicate	25.7	27.7	36.4	43.4	24.6	26.8	30.6	25.2	29.7	36.7
Magnesium silicate	18.8	22.7	22.2	22.5	21.8	20.3	21.5	14.3	15.2	21.6

* P = 40 pounds P_2O_5 per acre.

by all the plants grown on the magnesium silicated section is decreased, and correspondingly the magnesia content has increased. Shedd (17) and others have observed this fluctuation in the calcium and magnesium content of plants grown on soils receiving lime and magnesia applications.

AVAILABLE PHOSPHATE CONTENTS OF LIMED AND SILICATED SOILS IN RELATION TO YIELDS AND COMPOSITION OF PLANTS

If the increased yield of barley, rape, and sudan grass grown on the silicated soils is the result of increased availability of phosphates brought about by anion exchange reactions, manifestation of this reaction might possibly be

sought in the available phosphate content of the soil. Scarseth's results (15) indicate only a slight increase in the available phosphate content of silicated soils.

The data on the available phosphate content of the pot soils, by the Truog method (20), are presented in table 10. These recorded values represent the average available phosphate content of the soil before and after harvesting of the crops. This method of presentation of the results is used since only a very slight difference existed between the two values. Examination of the data in table 10 indicates that the difference in the available phosphate content of the silicated and the limed soils is so slight that not much significance can be attached to the values. It can therefore be seen that no relation exists between the yield and the available phosphate content of the soil. These data indicate that the availability of phosphates to the plants with reference to silicate applications is not reflected in chemical methods of determining availability. The data in table 10 were anticipated, since it was found earlier in this paper that the displacement of adsorbed phosphate ions occurs

TABLE 11

Electrodiffusible phosphate content of the high phosphate treated soils in relation to lime and silicate applications

SPECIAL TREATMENT	CHECK	ROCK PHOSPHATE	SUPERPHOSPHATE	DIAMMONIUM PHOSPHATE
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Hydrated lime	0.44	0.70	0.57	0.63
Calcium silicate	0.20	2.90	0.56	0.63
Magnesium silicate	0.13	1.06	0.41	0.41

only at high pH values. Differences do exist, however, between the available phosphorus contents of soil which received phosphate in the various forms. The values for rock phosphate are always greater than those for superphosphate or diammonium phosphate. This behavior has been noted by many investigators (5, 22).

The high phosphate treated soils of each special series after the removal of sudan grass were examined by a electrodialytic method for determining availability of phosphorus. The results, in table 11, emphasize the fact that the electrodiffusible phosphate content of the silicated soils, like the available phosphate content, is not greater than that of the limed soils, and that no correlation exists between yields and electrodiffusible phosphate content of the soils. It is interesting to note, however, that with rock phosphate and silicates the amount of electrodialyzable phosphate is greater than with lime. These results can best be explained in the light of Reifenberg's work (14), namely, that in the presence of silicates a peptization of the rock phosphate particles results in a corresponding increase in solubility.

SUMMARY

A study was made of the displacement of adsorbed phosphate ions from Colts Neck loam. The displacing anions used in the experiments were the hydroxyl, the silicate, the silicate at pH 7.0, and the sulfate. It was found that a replacement of the adsorbed ions results in solutions of anions that possess high pH values, namely, the hydroxyl and the silicate. Under this condition, degradation of the phosphated soil complex resulted. The displacement of the adsorbed phosphate ion seemed to be independent of the silicate-ion concentration but was dependent upon the pH. The displacing anions were classified into two groups with reference to the mobilization of the phosphate ions. Group one contained the hydroxyl and silicate ions, group two, the silicate at pH 7.0 and sulfate ions. The solutions of anions of the first group were associated with high pH values and large displacements of the adsorbed ion; the anions of the second group did not affect the release of the phosphate ion to any marked degree.

Crop yields from pot experiments are presented, and the chemical composition of the crops is discussed in the light of the fertilization with phosphates and silicates. A definite increase in crop yield was noted with barley and sudan grass when these crops were grown on soil to which calcium or magnesium silicate was added. No relation existed between the available phosphate contents of the soils and yields from the pots. Slight changes were noted in the chemical composition of the crops when grown on silicated soils. These changes were in the SiO_2, P_2O_5, CaO, and MgO content. A very marked absorption of silica by rape, barley, and sudan grass resulted when these plants were grown on silicated soils.

REFERENCES

(1) Butkewitsch, S., and Butkewitsch, W. W. 1925 The rôle of the Donnan membrane equilibrium in osmotic processes in the living cell: VI. *Biochem. Ztschr.* 161: 468–487.

(2) Demolon, A. 1933 Fixation et échange de certain anions par les colloides des sol. *Mezögazdasági Kutatasók* 6: 474–479.

(3) Demolon, A., and Bastisse, E. 1933 Fixation de la silice par l'argile colloidale. *Ann. Agron.* 1: 83–90.

(4) Fisher, R. A. 1929 A preliminary note on the effects of sodium silicate in increasing the yields of barley. *Jour. Agr. Sci.* 19: 132–139.

(5) Fraps, G. S. 1917 The availability of phosphoric acid in rock phosphate. Tex. Agr. Exp. Sta. Bul. 212.

(6) Gile, P. L., and Smith, J. G. 1925 Colloidal silica and the efficiency of phosphates. *Jour. Agr. Res.* 31: 247–260.

(7) Lemmermann, O. 1929 Zur Frage der Kieselsäurwirkung. *Ztschr. Pflanzenernähr. u. Düngung.* (A) 13: 28–39.

(8) Lemmermann, O., and Wiessmann, H. 1922 Die ertragssteigernde Wirkung der Kieselsäure bei Unzureichender Phosphorsäureenährung Pflanzen. *Ztschr. Pflanzenernähr. u. Düngung* (A) 1: 186–255.

(9) LEMMERMANN, O., AND WIESSMANN, H. 1924 Weitere Versuche die ertragssteigernde Wirkung der Kieselsäure bei Unzureichender der Phosphorsäuredüngung. *Ztschr. Pflanzenerähr. u. Düngung* (B) 3: 185–210.

(10) MATTSON, S. 1931 Laws of soil colloidal behavior: V. Ion adsorption and exchange. *Soil Sci.* 31: 311–331.

(11) NEMEC, A. 1928 Determination des besoins des sols en acide phosphorique d'apres la silicic soluble. *Compt. Rend. Acad. Sci.* [*Paris*] 186: 1060–1062.

(12) PARKER, F. W., AND FUDGE, J. F. 1927 Soil phosphorus studies: I. *Soil Sci.* 24: 109–117.

(13) RAVIKOVITCH, S. 1934 Anion Exchange: II. Liberation of the phosphate ions adsorbed by soils. *Soil Sci.* 38: 279–285.

(14) REIFENBERG, A. 1930 Über den Einfluss kolloider Kieselsäure auf die Assimilation der Phosphorsäure in Rohphosphaten. *Ztschr. Pflanzenernähr. u. Düngung* (A) 17: 1–11.

(15) SCARSETH, G. D. 1935 The mechanism of phosphate retention by natural aluminosilicate colloids. *Jour. Amer. Soc. Agron.* 27: 596–616.

(16) SCHOLLENBERGER, C. J. 1922 Silica and silicates in relation to plant growth and composition. *Soil Sci.* 14: 347–362.

(17) SHEDD, O. M. 1922 Effect of certain calcium compounds and other substances on the yield and calcium content of some crops. *Soil Sci.* 14: 233–246.

(18) THOMAS, W. 1930 The effect of colloidal silica on the absorption of phosphoric acid by plants. *Science* 71: 422–423.

(19) TOTH, S. J. 1937 Anion adsorption by soil colloids in relation to changes in free iron oxides. *Soil Sci.* 44: 299–314.

(20) TRUOG, E. 1930 The determination of the readily available phosphorus of soils. *Jour. Amer. Soc. Agron.* 22: 874–882.

(21) WEISER, V. L. 1933 Fixation and penetration of phosphates in Vermont soils. Vt. Agr. Exp. Sta. Bul. 356.

(22) WOLKOFF, M. 1924 Relative availability of the phosphorus of raw rock and acid phosphate in soils. *Soil Sci.* 17: 39–51.

PLATE 1

EFFECT OF LIME, MAGNESIUM SILICATE, AND CALCIUM SILICATE, WITH AND WITHOUT PHOSPHATE, ON YIELDS OF SUDAN GRASS

FIG. 1. Effect of lime and silicate on yields of sudan grass. 1—Magnesium silicate—no phosphate; 3—hydrated lime—no phosphate; 5—calcium silicate—no phosphate.

FIG. 2. Effect of lime and of magnesium and calcium silicates with rock phosphate on yields of sudan grass. 17—Calcium silicate—480 pounds P_2O_5 per acre as rock phosphate; 18—hydrated lime—480 pounds P_2O_5 per acre as rock phosphate; 19—magnesium silicate—480 pounds P_2O_5 per acre as rock phosphate.

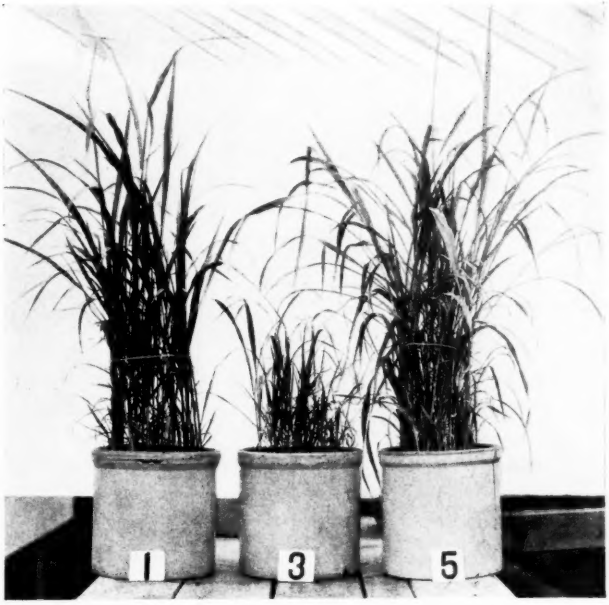


FIG. 1

FIG. 2

THE RÔLE OF POTASSIUM IN PLANTS: I. EFFECT OF VARYING AMOUNTS OF POTASSIUM ON NITROGENOUS, CARBOHYDRATE, AND MINERAL METABOLISM IN THE TOMATO PLANT[1]

MONROE E. WALL[2]

New Jersey Agricultural Experiment Station

Received for publication August 17, 1937

Studies involving the effect of potassium on the tomato and other plants have been made by previous investigators. Many of these investigations have dealt primarily with the effects of potassium deficiency on the metabolism of plants. Since many of these researches were carried out under different environmental conditions, with nutrient solutions of different composition, conflicting results have been obtained. Moreover, the interpretation of the results of chemical analyses, which are a criterion of plant metabolism, has also given rise to divergent hypotheses.

Little work has been done in the past in determining the effect of varying levels of potassium in tomato plant nutrition or their relation to the concentration of the other ions present in the nutrient solution. The object of this experiment, therefore, was to reconcile or explain the various hypotheses dealing with the effect of potassium deficiency on plants and to determine the effects of a high, medium, low, and minus potassium solution on the composition of the tomato plant, all other ions being kept constant as far as possible.

EXPERIMENTAL METHODS

Young tomato seedlings of the Rutgers variety, which had been grown in pots containing good loam soil, were washed free of soil and transplanted to glazed crocks containing washed white quartz sand. The plants received their nutrient supply by means of a constant drip method which was so regulated as to supply $3\frac{1}{2}$ liters of nutrient solution every 24 hours. The plants were set in sand March 20, 1936. At this time the seedlings were approximately 5 to 6 inches high, except those which were to receive the minus potassium solution, which were 8 to 9 inches high. The stems of the plants were rather hard and contained ample nitrates and appreciable starch. Two plants were set in each crock, and the crocks were divided into four series,

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, division of horticulture.

[2] The writer wishes to express appreciation to V. A. Tiedjens for invaluable advice and cooperation in conducting this investigation, to L. G. Schermerhorn for making necessary facilities available, to the American Potash Institute for financial assistance, and to C. S. Cathcart for the Kjeldahl determinations.

25 crocks to each series. The composition of the nutrient solutions used to grow the plant in the series is shown in table 1.

Sodium was substituted for potassium in order that the osmotic concentrations of all the solutions might be approximately the same. Moreover, it has been shown by Nightingale et al. (21) that sodium cannot replace potassium in the tomato plant. It should be noted that the terms "high," "medium," and "low" are arbitary designations for the potassium levels and have only comparative significance, being much higher than might be found in many soils. The plants received only distilled water for one week

TABLE 1

Nutrient solutions used in studying the relation of varying amounts of potassium to plant composition*

SALT OR ION	SERIES 1 HIGH POTASH	SERIES 2 MEDIUM POTASH	SERIES 3 LOW POTASH	SERIES 4 MINUS POTASH
	Partial volume molecular concentration			
NaH_2PO_4	.....	0.0045	0.00787	0.0090
KH_2PO_4	0.0090	0.0045	0.00113	.....
$Ca(NO_3)_2$	0.00675	0.00675	0.00675	0.00675
$MgSO_4$	0.00225	0.00225	0.00225	0.00225
	Parts per million of ions in solution			
Na	0	104	181	207
K	351	176	44	0
Ca	270	270	270	270
Mg	54	54	54	54
$PO_4 - P$	279	279	279	279
$NO_3 - N$	189	189	189	189
$SO_4 - S$	72	72	72	72

* Iron, boron, and manganese added in small amounts as necessary.

after being set in the crocks, after which nutrient solutions were supplied continuously for the duration of the experiment.

On April 8, 1936, the first signs of potassium deficiency appeared on the leaves of some of the plants in the minus potassium cultures. The young upper blades were yellow-green, and the upper petioles were dark purple as a result of the formation of anthocyanin pigments. Five days later the deficiency symptoms were very marked on most of the plants in this group. The upper section of the stem and the upper petioles had an intense purple color, and the upper blades were yellow-green and very mottled. The stems, moreover, were woody and hard. Microchemical tests at this time showed some nitrates and an abundant accumulation of starch. Many of the plants in this series had begun to set fruit, and growth had practically ceased. In contrast, the plants in the high, medium, and low potassium cultures were

succulent and growing vigorously. There was no anthocyanin formation, and both young and old blades were dark green.

The growth of the stems of the plants for the period from March 26 to April 13 was as follows:

DATE	AVERAGE HEIGHT			
	1 High	2 Medium	3 Low	4 Minus
	inches	*inches*	*inches*	*inches*
3/26/36	5	5	6	8½*
4/ 8/36	7½	6½	9½	11
4/13/36	12	10	13½	11

* The plants of the minus series were purposely selected to be larger than the plants of the other three series, so that there should be less discrepancy in plant size at the time of harvesting.

On April 20, 1936, half of the plants in each series were harvested for chemical analysis. Of the minus potassium plants, only those which showed early symptoms of deficiency were harvested. The plants of the other three series, at the time of harvest, exhibited a rather soft, succulent type of growth. At this time, the plants in series 3 (low potassium) were somewhat larger than those in series 1 (high potassium) and series 2 (medium potassium), which were approximately the same height. The average fresh weight of the whole stems of the plants at harvest was as follows: series 1, 46.2 gm.; series 2, 43.9 gm.; series 3, 51.7 gm.; and series 4, 23.1 gm.

By May 1, 1936, 10 days after the first harvest, many of the remaining deficient plants had undergone a marked transformation in external appearance. Resumption of growth was noticed. This new growth was very soft and succulent, in contrast to the hard previous growth, and the upper blades were dark green as compared with their earlier yellow-green appearance. Moreover, the anthocyanin color had completely disappeared. Shortly afterward, numerous rust-colored spots appeared on the blades of the lower leaves, closely following the vein pattern. The edges of these blades were curled upward. Dead areas appeared in these lower blades, and, finally, the entire leaf died. This first occurred at the base of the stem and continued progressively up the stem. A second harvest of the remaining plants in all the series for chemical analysis was made on May 20, 1936. At this time, series 1, 2, and 3, although much larger because of continuous growth, presented the same appearance as at the first harvest, whereas the leaves on the lower portion of the stems of the plants in the minus potash series had died and were not included.

It should be noted that, throughout, fruits were removed as soon as they appeared, in order to eliminate any effects on vegetative organs due to the removal of potassium by the fruit.

CHEMICAL METHODS

The plants at the first harvest were divided into upper and lower blades and whole stems, and at the second harvest there was a further fractionation into upper and lower stems. Fresh material was used for the determination of soluble nitrogen fractions. The plant material was well minced, and weighed portions were extracted with water by the method of Davidson et al. (3). The procedure outlined by Nightingale et al. (19, 21) was then followed for the determination of soluble, nitrate, and amino nitrogen, with the exception that nitrate nitrogen also included a small ammonia fraction, as did amino nitrogen. Protein nitrogen was determined as the difference between soluble and total nitrogen. Tissue for carbohydrate analyses was dried as recommended by Link (16). The procedure outlined by Nightingale et al. (19, 21) was then followed for the determination of reducing sugars, sucrose, and starch, with the exception that the Tompsett (29) method was used for the final determination of each fraction.

The dried tissue was also used for mineral analyses. One-gram samples were extracted with boiling water for one hour to determine soluble minerals. The aqueous extract was made up to a volume of 100 cc. Potassium was determined by precipitation as the insoluble potassium-sodium cobaltinitrite, the amount of nitrite being determined colorimetrically by application of the naphthylamine-sulfanilic acid reaction. Phosphorus was determined colorimetrically by a modification of the Bell and Doisy Method (1). Magnesium was separated from calcium and precipitated as magnesium-ammonium phosphate ($MgNH_4PO_4$), and the phosphate was determined as above. Calcium was precipitated as the oxalate, and titrated with $KMnO_4$ by means of a microburette. All the colorimetric determinations were made with a photoelectric cell. Full details of the procedure will be published in another paper.

For total minerals and ash, 1-gm. samples were ashed, and the ash was weighed. The ash was then taken up in hydrochloric acid, and after neutralization of the acid with NaOH, potassium, calcium, phosphorus, and magnesium were determined as above.

PRESENTATION OF RESULTS

The complete results of the chemical analyses are presented in tables 2 to 4 inclusive. In addition, some of the data are presented graphically in figures 1 and 2. The choice of a basis for presenting the results was difficult. The dry weight in series 4 fluctuated markedly in the course of the experiment, and the fresh weights of the various series were also somewhat variable because of slight wilting and the transition of the minus plants from a hard to a succulent type. The curves of data, calculated on green and dry weight bases, however, were similar. A rather puzzling feature is the discrepancy between results for protein nitrogen expressed on either a green or a dry weight basis and results based on distribution (quality) of nitrogen. These curves are

TABLE 2

Nitrogenous, carbohydrate, and mineral fractions of the upper blades of tomato plants grown with nutrient solutions containing different amounts

TABLE 2

Nitrogenous, carbohydrate, and mineral fractions of the upper blades of tomato plants grown with nutrient solutions containing different amounts of potassium

Potassium, *p.p.m*	351				176				44				None			
Ratio—Ca to K	0.77–1				1.53–1				6.14–1							
Harvest	April 20		May 20		April 20		May 20		April 20		May 20		April 20		May 20	
	% of G.M.*	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N
Nitrogenous fractions:																
Total organic N	0.750	100	0.693	100	0.784	100	0.783	100	0.968	100	0.786	100	0.901	100	0.992	100
Protein N	0.656	88	0.593	86	0.721	92	0.658	84	0.852	88	0.680	87	0.768	85	0.760	77
Soluble organic N	0.095	12	0.100	14	0.063	8	0.125	16	0.116	12	0.106	13	0.134	15	0.232	23
Amino N	.....	...	0.046	7	.....	...	0.052	7	.....	...	0.056	7	.....	...	0.101	10
Nitrate N†	0.043	6	0.027	4	0.049	6	0.030	4	0.043	4	0.031	4	0.063	7	0.062	6
Carbohydrate fractions:																
Dry matter	13.7	...	15.7	...	13.9	...	16.5	...	17.0	...	17.5	...	17.0	...	16.0	...
Reducing sugars	0.245	...	0.411	...	0.239	...	0.364	...	0.219	...	0.353	...	0.622	...	0.374	...
Sucrose	0.151	...	0.025	...	0.003	...	0.172	...	0.204	...	0.511	...	0.143	...	0.231	...
Starch and dextrins	0.189	...	0.323	...	0.101	...	0.464	...	0.352	...	0.586	...	0.237	...	0.262	...
Total carbohydrates	0.585	...	0.759	...	0.343	...	1.000	...	0.775	...	1.450	...	1.002	...	0.867	...
Mineral fractions:																
Total ash	1.567	...	1.562	...	1.689	...	1.716	...	1.919	...	1.876	...	1.779	...	1.453	...
Total K	0.515	...	0.384	...	0.451	...	0.376	...	0.437	...	0.337	...	0.163	...	0.197	...
Soluble Ca	0.165	...	0.195	...	0.185	...	0.262	...	0.235	...	0.297	...	0.248	...	0.157	...
Total Ca	0.296	...	0.275	...	0.338	...	0.311	...	0.405	...	0.413	...	0.345	...	0.248	...
Total Mg	0.062	...	0.066	...	0.081	...	0.064	...	0.073	...	0.072	...	0.097	...	0.066	...
Soluble PO_4	0.309	...	0.288	...	0.281	...	0.338	...	0.383	...	0.367	...	0.408	...	0.512	...
Total PO_4	0.370	...	0.328	...	0.367	...	0.396	...	0.481	...	0.419	...	0.578	...	0.757	...
Sol. Ca:Total Ca	0.55		0.71		0.55		0.84		0.58		0.72		0.72		0.63	
Sol. PO_4:Total PO_4	0.83		0.87		0.76		0.86		0.79		0.88		0.72		0.70	

* G.M. = green matter.

† NO_3—N on quality basis is calculated as per cent of total nitrogen.

TABLE 3

Nitrogenous, carbohydrate, and mineral fractions of the lower blades of tomato plants grown with nutrient solutions containing different amounts of potassium

Potassium, *p.p.m.*	351				176				44				None			
Ratio—Ca to K	0.77–1				1.53–1				6.14–1							
Harvest	April 20		May 20		April 20		May 20		April 20		May 20		April 20		May 20	
	% of G.M.*	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N
Nitrogenous fractions:																
Total organic N	0.564	100	0.249	100	0.504	100	0.412	100	0.575	100	0.396	100	0.515	100	....	....
Protein N	0.470	83	0.176	71	0.434	86	0.351	88	0.493	86	0.322	81	0.376	73	....	....
Soluble organic N	0.094	17	0.072	28	0.070	14	0.062	12	0.082	14	0.074	19	0.139	27	....	....
Amino N	.....	...	0.041	16	.....	...	0.029	7	.....	...	0.039	10	.....	...	....	....
Nitrate N†	0.075	12	0.037	13	0.071	12	0.040	9	0.069	11	0.039	9	0.063	11	....	....
Carbohydrate fractions:																
Dry matter	12.5	...	12.7	...	12.25	...	13.1	...	13.0	...	14.0	...	14.7	...	....	....
Reducing sugars	0.160	...	0.414	...	0.122	...	0.548	...	0.266	...	0.445	...	0.479	...	....	....
Sucrose	0.141	...	0.171	...	0.047	...	0.036	...	0.203	...	0.068	...	0.156	...	....	....
Starch and dextrins	0.144	...	0.226	...	0.139	...	0.164	...	0.179	...	0.192	...	0.244	...	....	....
Total carbohydrates	0.445	...	0.811	...	0.308	...	0.748	...	0.648	...	0.705	...	0.879	...	....	....
Mineral fractions:																
Total ash	2.166	...	2.612	...	2.183	...	2.688	...	2.016	...	3.262	...	2.388	...	....	....
Total K	0.375	...	0.342	...	0.368	...	0.295	...	0.285	...	0.217	...	0.181	...	....	....
Soluble Ca	0.264	...	0.449	...	0.262	...	0.603	...	0.267	...	0.678	...	0.327	...	....	....
Total Ca	0.572	...	0.705	...	0.627	...	0.847	...	0.665	...	0.941	...	0.757	...	....	....
Total Mg	0.094	...	0.091	...	0.093	...	0.088	...	0.084	...	0.108	...	0.144	...	....	....
Soluble PO_4	0.256	...	0.228	...	0.216	...	0.287	...	0.229	...	0.266	...	0.219	...	....	....
Total PO_4	0.311	...	0.366	...	0.343	...	0.354	...	0.347	...	0.449	...	0.463	...	....	....
Sol. Ca:Total Ca	0.44		0.64		0.42		0.71		0.40		0.72		0.43		....	
Sol. PO_4:Total PO_4	0.82		0.62		0.63		0.81		0.66		0.59		0.47		....	

* G.M. = Green matter.

† NO_3—N on quality basis is calculated as per cent of total nitrogen.

TABLE 4

Nitrogenous, carbohydrate, and mineral fractions of stems of tomato plants grown with nutrient solutions containing different amounts of potassium

Potassium, *p.p.m.*	351				176				44				None			
Harvest	April 20		May 20		April 20		May 20		April 20		May 20		April 20		May 20	
	% of G.M.*	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N
Nitrogenous fractions:																
Total organic N	0.284	100	0.161	100	0.264	100	0.208	100	0.284	100	0.208	100	0.264	100	....	....
Protein N	0.220	77	0.104	65	0.208	79	0.164	79	0.220	77	0.152	73	0.176	67	....	....
Soluble organic N	0.064	23	0.057	35	0.056	21	0.044	21	0.064	23	0.056	27	0.088	33	....	....
Amino N	.....	...	0.029	18	.....	...	0.020	10	.....	...	0.028	13	.....	...	....	....
Nitrate N†	0.040	14	0.028	17	0.040	15	0.024	12	0.040	14	0.028	13	0.048	18	....	....
Carbohydrate fractions:																
Dry matter	8.0	...	8.0	...	8.0	...	8.0	...	8.0	...	8.0	...	8.0	...	....	....
Reducing sugars	0.160	...	0.414	...	0.122	...	0.548	...	0.266	...	0.445	...	0.479	...	....	....
Sucrose	0.141	...	0.171	...	0.047	...	0.036	...	0.203	...	0.068	...	0.156	...	....	....
Starch and dextrins	0.144	...	0.226	...	0.139	...	0.164	...	0.179	...	0.192	...	0.244	...	....	....
Total carbohydrates	0.445	...	0.811	...	0.308	...	0.748	...	0.648	...	0.705	...	0.879	...	....	....
Mineral fractions:																
Total ash	1.166	...	1.612	...	1.183	...	1.688	...	1.016	...	2.262	...	1.388	...	....	....
Total K	0.375	...	0.342	...	0.368	...	0.295	...	0.285	...	0.217	...	0.181	...	....	....
Soluble Ca	0.264	...	0.449	...	0.262	...	0.603	...	0.267	...	0.678	...	0.327	...	....	....
Total Ca	0.572	...	0.705	...	0.627	...	0.847	...	0.665	...	0.941	...	0.757	...	....	....
Total Mg	0.094	...	0.091	...	0.093	...	0.088	...	0.084	...	0.108	...	0.144	...	....	....
Soluble PO_4	0.256	...	0.228	...	0.216	...	0.287	...	0.229	...	0.266	...	0.219	...	....	....
Total PO_4	0.311	...	0.366	...	0.343	...	0.354	...	0.347	...	0.449	...	0.463	...	....	....
Sol. Ca:Total Ca	0.44		0.64		0.42		0.71		0.40		0.72		0.43		....	
Sol. PO_4:Total PO_4	0.82		0.62		0.63		0.81		0.66		0.59		0.47		....	

* G.M. = Green matter.

† NO_3—N on quality basis is calculated as per cent of total nitrogen.

† NO_3—N on quality basis is calculated as per cent of total nitrogen.

TABLE 4

Nitrogenous, carbohydrate, and mineral fractions of stems of tomato plants grown with nutrient solutions containing different amounts of potassium

Potassium, *p.p.m.*	351						176						44						None					
Ratio—Ca to K	0.77–1						1.53–1						6.14–1											
	Whole stem		Upper stem		Lower stem		Whole stem		Upper stem		Lower stem		Whole stem		Upper stem		Lower stem		Whole stem		Upper stem		Lower stem	
Harvest	April 20		May 20		May 20		April 20		May 20		May 20		April 20		May 20		May 20		April 20		May 20		May 20	
	% of G.M.*	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N	% of G.M.	% of total N
Nitrogenous fractions:																								
Total organic N	0.180	100	0.264	100	0.358	100	0.190	100	0.213	100	0.351	100	0.190	100	0.242	100	0.382	100	0.255	100	0.337	100	0.446	100
Protein N	0.096	53	0.201	76	0.199	56	0.116	61	0.127	60	0.194	55	0.097	51	0.157	69	0.179	47	0.149	58	0.174	51	0.201	45
Soluble organic N	0.084	47	0.062	24	0.159	44	0.075	39	0.086	40	0.157	45	0.093	49	0.084	31	0.203	53	0.107	42	0.163	49	0.245	55
Amino N	.....	...	0.043	16	0.096	27	.....	...	0.052	24	0.102	29	.....	...	0.056	23	0.111	29	.....	...	0.104	31	0.126	28
Nitrate N†	0.114	39	0.045	15	0.098	22	0.101	35	0.050	19	0.104	23	0.118	38	0.053	18	0.095	20	0.104	30	0.107	24	0.090	17
Carbohydrate fractions:																								
Dry matter	9.3	...	11.2	...	17.2		9.0	...	10.5	...	16.6	...	10.6	...	12.0	...	18.0	...	12.8	...	11.2	...	15.0	...
Reducing sugars	0.609	...	1.156	...	0.860	...	0.705	...	1.058	...	0.634	...	0.979	...	1.373	...	0.589	...	0.874	...	0.733	...	0.733	...
Sucrose	0.243	...	0.392	...	0.979	...	0.326	...	0.748	...	1.306	...	0.538	...	0.786	...	1.667	...	1.078	...	0.582	...	0.635	...
Starch and dextrins	0.110	...	0.075	...	0.816	...	0.107	...	0.219	...	0.869	...	0.221	...	0.292	...	0.935	...	0.979	...	0.161	...	0.171	...
Total carbohydrates	0.962	...	1.623	...	2.655	...	1.138	...	2.025	...	2.809	...	1.738	...	2.451	...	3.191	...	2.931	...	1.476	...	1.539	...
Mineral fractions:																								
Total ash	1.386	...	1.047	...	1.678	...	1.326	...	0.901	...	1.591	...	1.261	...	1.018	...	1.631	...	1.133	...	0.876	...	1.009	...
Total K	0.493	...	0.378	...	0.368	...	0.411	...	0.314	...	0.375	...	0.296	...	0.289	...	0.279	...	0.124	...	0.119	...	0.129	...
Soluble Ca	0.086	...	0.094	...	0.164	...	0.073	...	0.119	...	0.181	...	0.095	...	0.114	...	0.175	...	0.114	...	0.089	...	0.150	...
Total Ca	0.153	...	0.119	...	0.309	...	0.173	...	0.121	...	0.329	...	0.232	...	0.154	...	0.407	...	0.242	...	0.186	...	0.335	...
Total Mg	0.054	...	0.035	...	0.083	...	0.050	...	0.036	...	0.079	...	0.049	...	0.041	...	0.090	...	0.068	...	0.044	...	0.044	...
Soluble PO_4	0.186	...	0.221	...	0.333	...	0.198	...	0.178	...	0.290	...	0.223	...	0.170	...	0.342	...	0.263	...	0.221	...	0.282	...
Total PO_4	0.234	...	0.232	...	0.334	...	0.208	...	0.204	...	0.311	...	0.223	...	0.207	...	0.387	...	0.275	...	0.275	...	0.307	...
Sol. Ca:Total Ca	0.56		0.78		0.53		0.42		0.99		0.55		0.41		0.74		0.43		0.47		0.48		0.45	
Sol. PO_4:Total PO_4	0.79		0.99		0.99		0.99		0.87		0.93		1.00		0.63		0.89		0.91		0.80		0.91	

* G.M. = green matter.

† NO_3—N on quality basis is calculated as per cent of total nitrogen.

very similar for series 1, 2, and 3. For series 4, however, results on a green or a dry weight basis show the protein fraction to be higher than that for the other series except in the upper and lower leaves of the first harvest. The same data expressed on a quality basis show protein in series 4 to be generally lower than that in all the other series. Results for soluble organic nitrogen

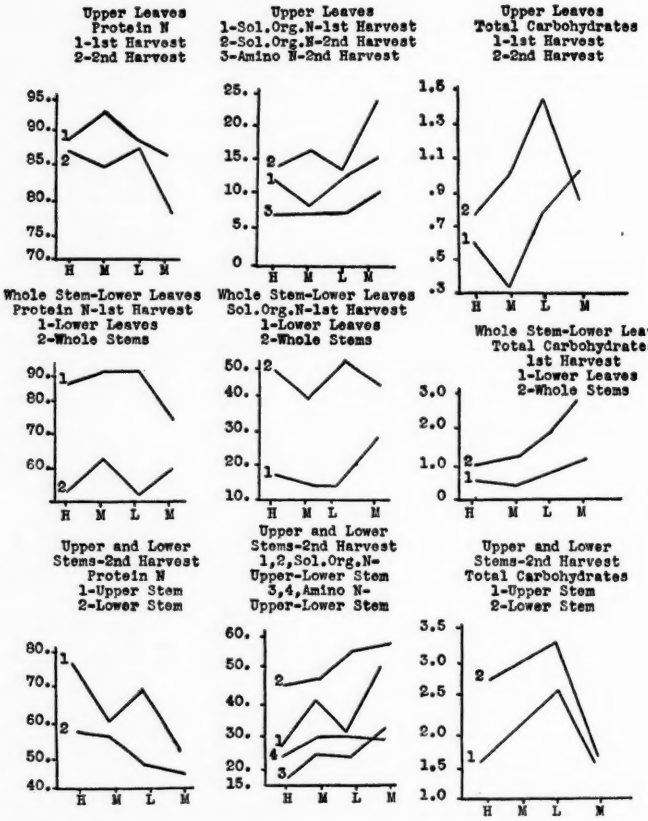


FIG. 1. NITROGENOUS AND CARBOHYDRATE FRACTIONS OF TOMATO PLANTS GROWN WITH NUTRIENT SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF POTASSIUM

are similar when plotted on either basis. Since the deficient plants grew but little in comparison to the plants of the other series, it is possible that the same or a lower amount of protein or any other analytical fraction in the minus series could be concentrated in a smaller area. This would account for the high values for protein in series 4. Gregory (8) accounts for the increase in total nitrogen in potassium deficient barley plants on this basis.

Interpretations of the nitrogen data will, therefore, be made on the quality basis, all the other data being discussed on the fresh weight basis. There were few large, significant differences in protein and soluble organic nitrogen

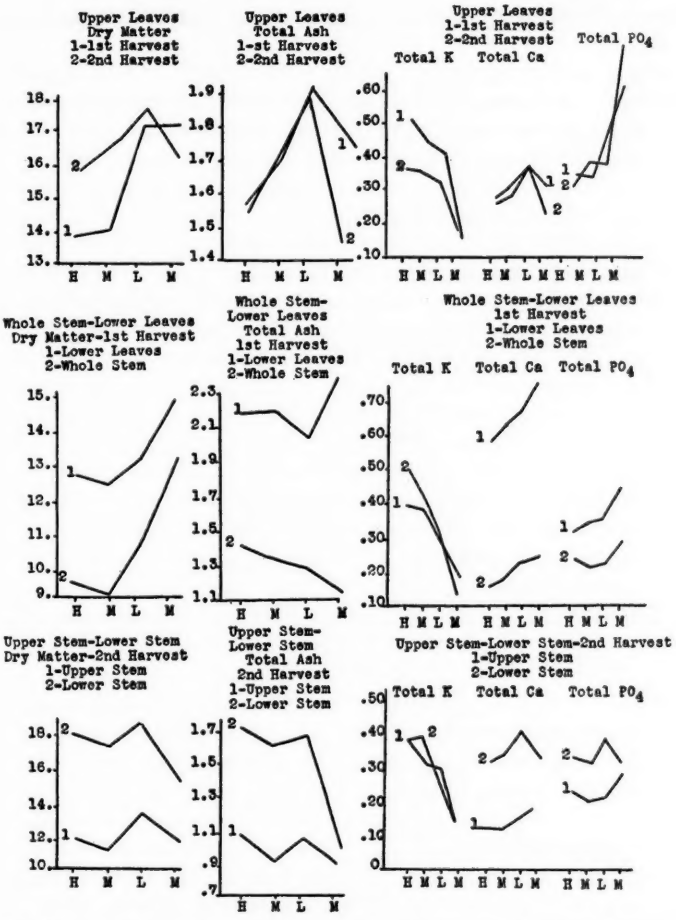


FIG. 2. MINERAL FRACTIONS OF TOMATO PLANTS GROWN WITH NUTRIENT SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF POTASSIUM

between series 1, 2, and 3. Series 4, as shown in figure 1, was generally lower in protein nitrogen and higher in soluble organic nitrogen than the other series.

The accumulation of carbohydrates in the potassium deficient plants at the first harvest and the subsequent large decrease in carbohydrates in these plants at the second harvest are clearly shown in figure 1. These results

follow the dry weight curves (fig. 2) rather closely. The high carbohydrate content at both harvests of the plants in series 3 is of considerable interest. There were only small differences in carbohydrate content between series 1 and 2.

These results will be referred to again and amplified in the discussion of the data.

Tables 2 to 4 inclusive also present the ratio of soluble to total calcium and phosphorus. With the exception of series 4 there is but little difference between the various nutrient treatments. In comparison with the other series, the potassium deficient series had a higher ratio of soluble to total calcium in the upper leaves at the April harvest and a lower ratio in the upper leaves and in the upper and lower stems at the May harvest. It is interesting to note, in series 1, 2, and 3, the variation in proportion of soluble minerals, which apparently depends on the age and part of the plant considered. In the plants of these series at the first harvest, calcium averaged about 55 per cent soluble in the upper leaves, 40 per cent soluble in the lower leaves, and 45 per cent soluble in the whole stems; whereas at the second harvest, calcium was about 70 per cent soluble in both upper and lower leaves, 75 per cent soluble in upper stems, and 50 per cent soluble in the lower stems. Phosphate did not vary much in any particular part of the plants in series 1, 2, and 3, although the stems were always higher in soluble phosphate than were the leaves. The lower leaves of all series at both harvests were much higher in total calcium than any other part of the plant. There was no such difference in phosphate or magnesium content.

DISCUSSION OF RESULTS

Potassium deficiency symptoms

There are rather marked external symptoms of potassium deficiency in the tomato. These may be divided into two stages. The first stage (which under some conditions may be absent) is associated with a hard type of plant accompanied by chlorosis and mottling of the upper blades. There may be an intense anthocyanin pigmentation of the upper portion of the stem and petioles. Growth is markedly retarded, elongation may almost cease, and the stem is much smaller in circumference than the stem of a normal plant. Fruit is usually set earlier in the deficient plants, and other symptoms (21), which will not be discussed here, are also associated with the fruit. In the second stage of potassium deficiency, the tomato plant becomes succulent, the leaves become dark green, and the stem elongates rapidly. This secondary new growth develops 1 to 3 weeks after initiation of the first deficiency symptoms. It is associated with marked injury to the leaves, commencing as a rule with small brown peppery spots more or less along the veins on the older lower leaves. The edges of many of the leaves are curled upward as a result of uneven growth. All but the youngest leaves will exhibit injury to some

degree. The spots soon enlarge and then form dead areas with considerable dying of tissue at the margins of the leaves. Nightingale (21) believes that the peppery spots in potassium deficient leaves are due to iron injury. Iron injury produced large black areas superficially resembling mosaic in the tomato leaves. Both stages give rise to marked histological differences, which have been well discussed by Nightingale et al. (21).

The tomato plants of this investigation and those studied by Nightingale were grown in the same greenhouse and under similar conditions. The symptoms of deficiency were in most cases similar. Investigations by other workers (2, 8, 10, 12) with tomatoes and other plants, however, reveal some discrepancies. Janssen and Bartholomew (13) report potassium deficient tomatoes to be dark green, with purple-brown stems, the lower leaves dying first, but they do not report the early symptoms mentioned above. Gregory (8) mentions two types of potassium deficiency in barley. One is associated with low potassium and calcium and a high sodium supply in the nutrient medium. This is correlated with succulence and very rapid death of the leaves. The other type of deficiency occurs with high calcium and low potassium, sodium, and phosphorus. This type of nutrient apparently causes a cessation of meristematic activity and growth. Gregory extends this observation to include all plants, but there seems to be considerable evidence against any such generalization. The tomato plants of this investigation, like those of Nightingale, were supplied with a nutrient containing high sodium and medium to high calcium and phosphorus. These plants exhibited both types of deficiency mentioned by Gregory. Hartt (10) working with sugar cane found that the same type of deficiency, manifested by depressed growth and discoloration of the blades, occurred whether or not sodium was substituted for potassium. In Hartt's experiments, calcium, phosphorus, and sodium (when supplied) were rather high. In view of these facts, the author believes that environmental conditions such as temperature and light coupled with derangements in internal metabolism due to potassium deficiency may be primarily responsible for deficiency symptoms. Moreover, the low calcium and phosphorus nutrient mentioned by Gregory may approach deficiency and thus markedly alter the metabolism.

The effect of environmental conditions is very important in any discussion on potassium deficiency. Tomato plants grown in winter with high calcium sodium, and phosphorus showed no hardening (accumulation of carbohydrates) or cessation of elongation. Plants grown in early summer which were shaded showed the same symptoms. In other words, light, inasmuch as it affected the internal metabolism of the plant, affected the external deficiency symptoms. It would seem probable that, in the case of potassium deficiency, lowering of temperature could produce a hard plant in any season, due to accumulation of carbohydrates. Experimental evidence to this effect has been found at this laboratory.

Nitrogen metabolism

Observations by the various workers on the effect of potassium on the nitrogen metabolism of green plants are not in agreement. Janssen and Bartholomew (13) found that deficiency of potassium caused an increase in total nitrogen in the tomato plant. Nightingale (21) could find little difference in nitrogen content or nitrogenous fractions between controls and deficient plants. Burrell (2) working with soybeans found that potassium deficiency resulted in a decrease of protein nitrogen and an increase in the amino and amide nitrogen. Later work by Janssen et al. (14) with the tomato presented like results. Similar results have been reported by Hartt (11) working with sugar cane. The general conclusion of these workers indicates that potassium in some way affects the formation of organic nitrogen. Some (21) believe, however, that the initial reduction of nitrate to organic forms of nitrogen is affected, and others (10) believe that the formation of protein nitrogen from soluble organic forms is interfered with. On the other hand, Richards and Templeman (24), who investigated the effect of potassium deficiency on barley, reported a normal protein content at the time of emergence of the leaves and a rapid breakdown at the time of senescence. Hence, they conclude that potassium is not essential to protein synthesis but is essential for the maintenance of the protoplasmic complex. It is undoubtedly true that under conditions of severe potassium deficiency protoplasm will break down, resulting in the rapid death of leaves. Under such conditions, carbohydrates are very low. Hence the direct cause of senescence may be due to a decrease in carbohydrate content resulting in a drop in respiration. When this occurs, protein will be hydrolyzed and the soluble products translocated primarily to the meristematic areas. If potassium has no effect on protein synthesis, it is difficult to explain why these breakdown products should not be resynthesized to protein in the meristematic areas, unless the rate of proteolysis is much more rapid than the rate of synthesis. This last hypothesis does not seem probable, because abnormally rapid proteolysis usually takes place only in senescent tissues. The carbohydrate accumulation in the minus plants of the first harvest is also evidence that some link in the chain of protein synthesis is broken. This point will be discussed later in some detail. The results of this experiment show that protein in the meristematic areas, that is, upper leaves and upper stems, was consistently lower than the controls. If one assumes that below a certain minimum concentration of potassium, protein synthesis is retarded, the decrease in protein content of the meristematic areas in this investigation may be accounted for. Nightingale (21) found a much higher proportion of potassium in the meristematic portions of potassium deficient tomatoes than in other regions. Penston (22) and James and Penston (12) found potassium abundant in the meristematic regions of the potato, and noted that both protein and potassium had the same distribution. There is scarcely any potassium in dead

tomato leaves (21), the conclusion being that it is translocated to meristematic regions.

There is still further evidence that potassium may be directly concerned in protein synthesis. Hartt (11) found a decrease in peptase activity of potassium deficient sugar cane. In conjunction with this statement, the accumulation of carbohydrates in the minus plants of the April harvest should be noted, although carbohydrates decrease in plants of the second harvest, for causes which will be discussed later. Since the synthesis of protein involves a chain of reaction in which carbohydrates are involved, any break in this chain could cause accumulation of carbohydrates. These reactions

$$\underset{\text{I}}{NO_3 \leftrightarrows NO_2 \leftrightarrows NH_3} \cdot \underset{\text{II}}{NH_3 + \text{Hexose} \leftrightarrows R\text{–}NH_2COOH}$$

may be postulated as: $\underset{\text{III}}{(R\text{–}NH_2COOH)_x \leftrightarrows \text{Protein}}$. All of these products, except protein, are soluble. Hence the law of mass action, $A + B \leftrightarrows C + D$, can be provisionally applied to reactions I and II. In other words, if amino acids should accumulate, the tendency would be to shift the reaction back to the ammonium stage. It has not been shown that this reaction is reversible to the nitrate form in plants. It is not necessary, however, for such a reaction to reverse itself in order that nitrates may accumulate in plants, for as long as the forward reaction $NO_3 \leftrightarrows NO_2$ is halted, the nitrate concentration will rise, since absorption will not be halted. Carbohydrates would accumulate for the same reason. This may account for the accumulation of carbohydrates and retention of nitrates in the minus potassium plants. Processes of translocation and partial transformation to protein obscure the application of this law. Nightingale (21) has shown that potassium deficient plants shifted to a minus nitrate solution retained their initial nitrate content. This seems to be an example of the law of mass action operating in plants, if the reducase explanation is incorrect. It would seem then that soluble organic fractions in potassium deficient plants increase partly because of hydrolysis of protein. Here potassium is not directly concerned in the nitrogen metabolism. The accumulation of these fractions whether of hydrolytic origin or not, would seem to be due, however, to a retardation of protein synthesis. If potassium is viewed as a catalyst for this reaction, then it is obvious that within limits the greater the concentration of catalyst, the greater the speed of reaction, and vice versa.

No mention has as yet been made of the effect of potassium on reducase activity. Reducase apparently reduces nitrate to nitrite and ammonia (5). Reducase activity has been found to be much lower than normal in potassium deficient plants (21). For this reason it is claimed that the utilization of nitrates by the plant is affected by potassium and that this is the cause of carbohydrate accumulation. Yet soluble organic fractions in these plants and in those of other investigations (2, 10, 13, 24) are invariably higher than

in normal plants, in both slight and extreme deficiency. If nitrates, however, are not reduced, it is difficult to account for such an accumulation of soluble organic nitrogen, especially in the early stages of potassium deficiency where proteolytic breakdown is not taking place rapidly. Moreover, the increase in soluble organic nitrogen due to phosphorus deficiency is probably not due to proteolysis (24). On this basis, it would seem that nitrate assimilation may not be directly affected by potassium deficiency. A brief review of the effect of various ions on reducase activity shows that potassium (21), phosphorus (6), and calcium (18) deficiencies all lower reducase activity. Tiedjens (27) reports that plants supplied with ammonium nitrogen show but little reducase activity. Eckerson (5) has shown that nitrate deficient plants have higher reducase activity than do controls. It should be noted that all the treatments, with the exception of calcium deficiency, whereby reducase activity is lowered have one result in common—a great increase of soluble organic nitrogen. On the other hand, where soluble organic nitrogen is low, in nitrate deficient plants, reducase activity is high. Until more experimental evidence[3] is presented, it is impossible to say with certainty that a high concentration of soluble organic nitrogen may depress reducase activity. It does not seem probable, however, that so many diverse ions as K, Ca, PO_4, NH_4, and NO_3 should by their presence or absence affect an enzyme-like substance. The foregoing hypothesis in no way denies that nitrates may be reduced by reducase, but it does cast doubt upon the probability of all these ions being directly involved in reducase activity and hence in nitrate assimilation. It should be recalled again that accumulation of carbohydrates and nitrates can be postulated upon a mass action basis, if any soluble organic fraction accumulates. Hence the necessity of postulating any alteration in nitrate reduction due to reducase inactivation can be eliminated. Richards and Templeman (24) have expressed similar views. The accumulation of carbohydrate together with an increase of soluble organic nitrogen is not incompatible, if a large amount of carbohydrate is present, because it can be calculated that 9 mgm. of a 6-carbon organic acid requires only 1 mgm. of nitrogen to form an amino acid. Hence a slight reversal of the forward reaction in the case of nitrogen might cause an enormous accumulation of carbohydrate. It is possible then that the changes in composition attributed to the cessation of reducase activity in potassium deficient plants may actually be due to a decrease in rate of protein synthesis at the amino acid stage, and the cessation of reducase an indirect rather than a direct result of potassium deficiency. Increasing the amount of potassium after deficiency levels have been passed does not markedly affect the nitrogen metabolism of the tomato. The trends seem to indicate that series 3 is higher than series 1 and 2 in percentage of total organic nitrogen. Gassner and Goeze (7) have reported that low K and high N have the same effect. The results of this experiment, as far as series 1, 2, and 3 are concerned, seem to verify this conclusion.

[3] Work on this phase is being conducted at this laboratory.

Carbohydrate metabolism

There are conflicting reports in the literature upon the effect of potassium on carbohydrate metabolism. Some workers report carbohydrate accumulation (21) in the case of deficiency, whereas others report the reverse (8). Environmental conditions and type of deficiency (early or extreme) will markedly influence the results. Generally, carbohydrates will decrease in severe and long-standing potassium deficiency. The minus potassium plants of this investigation showed a marked accumulation of carbohydrate (hexose, sucrose, and starch) at the April harvest. This was undoubtedly due to lack of utilization of carbohydrates in the formation of soluble organic nitrogen, primarily amino acid. The cause of this carbohydrate accumulation has already been discussed. In plants from the May harvest, starch and sucrose had fallen to very low values, although hexose was still high. Gregory and Richards (9) and Richards (23) have shown that potassium deficiency increases respiration and decreases assimilation. Richards reports that respiration increases with increasing potassium deficiency but falls with extreme potassium starvation. Since respiration is dependent to some extent on sugar content, and since in extreme potassium starvation sugar content is low, it is obvious why respiration will eventually decrease in extreme potassium deficiency. The increased respiration of potassium deficient plants may be due to the effects of the high amino acid content (25, 26). Since assimilation is also low in potassium deficiency, the increased respiration will eventually greatly reduce the total carbohydrate content of the plant, even though, as shown by the data of this investigation, carbohydrates may not be utilized to any great degree, because of the decrease in protein formation. The comparatively high hexose content in the deficient plants of the second harvest may represent the hydrolysis products of starch and sucrose. That starch may still be synthesized seems evident from the work of Doby and Hibbard (4) who found amylose higher than normal in potassium deficient sugar beets, and of Hartt (11) who found it higher in potassium deficient sugar cane.

Carbohydrate data for series 1, 2, and 3 reveal interesting comparisons. Series 3 is higher in total carbohydrate than series 2, which in turn is higher than series 1. This hold true, in general, for both harvests. Gassner and Goeze (7) give evidence of a low but optimum potassium supply below which assimilation decreases rapidly and above which it declines gradually to the highest potassium dosage. These results are apparently identical with those obtained in the present investigation as shown by data in tables 2 to 4. The plants of series 3 were the best plants in regard to general appearance, height, fresh weight, and dry weight. This result has been confirmed in an investigation to be published later where the tomatoes were allowed to fruit. The fruit in this case was much larger and more abundant than that of controls containing a much higher amount of potassium. It is of interest to note that this

quantity of potassium (44 p.p.m.) is not only an optimum[4] supply under the environmental conditions of this experiment, but also practically a minimum supply. Later work by the author[5] has shown that under these conditions the potassium supply cannot be reduced much below 44 p.p.m. before tomatoes in sand culture will show signs of deficiency.

Whether this stimulation of assimilation and consequent increase in dry weight and carbohydrate production are due to the stimulating effect of a low potassium nutrient or to the effect of interionic relationship is unknown. Lundegardh (17) reports that antagonism between potassium and calcium is very prominent. He says,

> "... the remarkable antagonistic effect of K toward Ca is analagous with the exchange of K and Ca in soil colloids. K is attracted more by the negatively charged colloids than Ca. In tissues the antagonistic effect of K increases as a consequence of its more rapid movement. The degree of antagonism varies of course with the ratio of concentrations of the two ions. With the same external concentration K accumulates in the plant much more than Ca."

The ratio of Ca to K in series 3 (p.p.m. of external supply) was 6 to 1. Experiments with onions[6] showed a decrease in dry weight, as the ratio of calcium to potassium was decreased. The work of Tiedjens (28) with tomatoes shows the same result both for dry weight and for carbohydrates. Moreover, in Tiedjens' work potassium was kept at a high level and calcium was increased. Hence, the antagonistic action of Ca and K may affect assimilation.

The effect of environmental conditions, particularly light, on external symptoms of potassium deficiency has been discussed previously. This is undoubtedly associated with the carbohydrate metabolism of potassium deficient plants. Under conditions of reduced light, not enough carbohydrate is formed to accumulate, especially if reduced assimilation and increased respiration occur. On the other hand, favorable light conditions may, in the first stages of potassium deficiency, form an abundance of carbohydrate which may then be accumulated. This accumulation may favor the formation primarily of storage protein (20), and hence growth is checked. Temperature effects could probably cause the same thing.

The death of the potassium deficient plants seems to be due primarily to the low carbohydrate content of these plants. Whether potassium or some radioactive isotope is concerned in the assimilation and elaboration of CO_2 to carbohydrates cannot be determined with present methods and limited knowledge of photosynthetic processes. There may also be the possibility that the antagonistic effects of ions such as Ca and K are necessary to keep protoplasm in condition to carry on photosynthesis.

[4] The supply under these conditions of nutrition may be somewhat higher for an optimum, but not greatly higher.

[5] Unpublished data.

[6] Unpublished data by the author.

Mineral metabolism

Series 3 (44 p.p.m. of K) was slightly higher than series 1 and 2 in total ash except in the stems, and in individual minerals except potassium. This may be explained on the basis of the lower potassium content of the tomatoes of series 3. Since potassium is supposedly more mobile than other ions, a decrease in potassium would favor increased absorption of the other ions. Series 4 fluctuated somewhat. That series 3 and 4 did not have much larger total ash contents than series 1 and 2, may have been due to the substitution of sodium for potassium, inasmuch as sodium is much less mobile than potassium. The very high phosphate content has also been found by Johnston and Hoagland (15) in potassium deficient tomatoes and by Hartt (10) in potassium deficient sugar cane. It is possible that potassium deficiency may lead to changes in the permeability of cells to phosphates and to other solutes. At times, soluble calcium and phosphate were more abundant in series 4 than in the other series, and at other times less abundant. Calcium was usually less soluble. At present no explanation can be made of these results, although the insolubility of calcium in the meristematic upper stems of series 4 seems to be significant and may reveal a derangement in the calcium binding power of the protein. The varied distribution in the plant and solubility of the ions, especially calcium, are of interest, but they have no immediate bearing on this investigation. As was to be expected, the potassium content of the plants was usually proportional to the potassium content of the nutrient. But the internal ratio of potassium to the other ions varied greatly, depending on the portion of the plant analyzed. This is probably due to the greater mobility and solubility of potassium. The potassium content of the minus plants was rather low and corresponded somewhat to other investigations.

SUMMARY

The symptoms of potassium deficiency are discussed, and evidence is presented that environmental conditions in relation to changes in internal metabolism due to potassium deficiency are primarily responsible for the different deficiency symptoms that have been noted.

Deficiency in potassium seems to curtail protein synthesis. This seems to occur in the stage amino acid-protein after amino acids have been formed.

Accumulation of nitrates and carbohydrates is explained on a partial mass action effect rather than on a direct retardation in nitrate assimilation.

The final decrease in carbohydrates in potassium deficient plants is due to the effects of a decrease in assimilation and an increase in respiration.

Potassium deficient plants show an increase in the absorption of other ions, particularly phosphate, although this is partially masked by the effects of substituting sodium for potassium.

Preliminary evidence is given of a low concentration of potassium which

is not only an optimum supply, but is also close to a minimum supply for tomatoes in sand culture under the experimental conditions.

The possible bearing of the antagonism of calcium to potassium in relation to carbohydrate assimilation and growth is discussed.

REFERENCES

(1) Bell, R. D., and Doisy, E. A. 1920 Rapid colorimetric methods for the determination of phosphorus in urine and blood. *Jour. Biol. Chem.* 44: 55–67.

(2) Burrell, R. C. 1926 Effect of certain deficiencies on nitrogen metabolism of plants. *Bot. Gaz.* 82: 320–329.

(3) Davidson, O. W., Clark, H. E., and Shive, J. W. 1934 Preparation of aqueous extracts of soluble nitrogen from plant tissues. *Plant Physiol.* 9: 817–822.

(4) Doby, G., and Hibbard, R. P. 1926 Verhalten insbesondere Ionaktivierung von Pflanzenenzymen in Abhangigkeit von der Ernahrung. *Biochem. Ztschr.* 73: 107–161.

(5) Eckerson, S. H. 1924 Protein synthesis by plants. I. Nitrate reduction. *Bot. Gaz.* 77: 377–391.

(6) Eckerson, S. H. 1929 Influence of phosphorus deficiency on the metabolism of tomato plants. *Amer. Jour. Bot.* 16: 852–853.

(7) Gassner, G., and Goeze, G. 1934 Assimilation behavior, chlorophyll content, and transpiration rate of cereal leaves with special reference to potassium and nitrogen. *Ztschr. Bot.* 27: 257–340.

(8) Gregory, F. G. 1937 Mineral nutrition of plants. *Ann. Rev. Biochem.* 6: 557–578.

(9) Gregory, F. G., and Richards, F. J. 1929 Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. *Ann. Bot.* 43: 119–161.

(10) Hartt, C. E. 1934 Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. *Plant Physiol.* 9: 399–452.

(11) Hartt, C. E. 1934 Some effects of potassium upon the amounts of protein and amino forms of nitrogen, sugars, and enzyme activity of sugar cane. *Plant Physiol.* 9: 452–491.

(12) James, W. O., and Penston, N. L. 1933 Studies of the physiological importance of the mineral elements in plants. IV. The quantitative disbribution of potassium in the potato plant. *Ann. Bot.* 47: 279–293.

(13) Janssen, G., and Bartholomew, R. P. 1929 The translocation of potassium in tomato plants in its relation to their carbohydrate and nitrogen distribution. *Jour. Agr. Res.* 38: 447–465.

(14) Janssen, G., Bartholomew, R. P., and Watts, V. M. 1934 Some effects of nitrogen, phosphorus, and potassium on the composition and growth of tomato plants. Ark. Agr. Exp. Sta. Bul. 310.

(15) Johnston, E. S., and Hoagland, D. R. 1929 Minimum potassium level required by tomato plants grown in water cultures. *Soil Sci.* 27: 89–109.

(16) Link, K. P. 1923 Effects of the method of desiccation on the carbohydrates of plant tissue. *Jour. Amer. Chem. Soc.* 45: 439–447.

(17) Lundegårdh, H. 1934 Mineral nutrition of plants. *Ann. Rev. Biochem.* 3: 485–498.

(18) Nightingale, G. T., Addoms, R. M., Robbins, W. R., and Schermerhorn, L. G. 1931 Effects of calcium deficiency on nitrate absorption and on metabolism in the tomato. *Plant Physiol.* 6: 605–630.

(19) Nightingale, G. T., and Robbins, W. R. 1928 Some phases of nitrogen metabolism in polyanthus narcissus. N. J. Agr. Exp. Sta. Bul. 472.

(20) Nightingale, G. T., Schermerhorn, L. G., and Robbins, W. R. 1928 The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. N. J. Agr. Exp. Sta. Bul. 461.

(21) NIGHTINGALE, G. T., SCHERMERHORN, L. G., AND ROBBINS, W. R. 1930 Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. N. J. Agr. Exp. Sta. Bul. 499.

(22) PENSTON, N. L. 1931 Studies of the physiological importance of the mineral elements in plants. III. A study by microchemical methods of the distribution of potassium in the potato plant. *Ann. Bot.* 45: 673–691.

(23) RICHARDS, F. J. 1932 Physiological studies in plant nutrition. III. Further studies of the effect of potash deficiency on the rate of respiration in barley. *Ann. Bot.* 46: 367–388.

(24) RICHARDS, F. J., AND TEMPLEMAN, W. G. 1936 Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. *Ann. Bot.* 50: 367–402.

(25) SCHWABE, G. 1932 Effect of amino acids on the oxygen consumption of submerged plants. *Protoplasma* 16: 397–451.

(26) SPOEHR, H. A., AND MCGEE, N. J. M. 1924 Studies in Plant Respiration and Photosynthesis. Carnegie Inst., Washington.

(27) TIEDJENS, V. A., AND BLAKE, M. A. 1932 Factors affecting the use of nitrate and ammonium nitrogen by apple trees. N. J. Agr. Exp. Sta. Bul. 547.

(28) TIEDJENS, V. A., AND SCHERMERHORN, L. G. 1936 Available calcium a factor in salt balance for vegetable crops. *Soil Sci.* 42: 419–433.

(29) TOMPSETT, S. L. 1930 Determination of blood sugar. I. Critical analysis of the reduction of alkaline copper reagents by glucose and other substances. *Biochem. Jour.* 24: 1148–1163.

BOOK REVIEWS

Micropedology. By WALTER L. KUBIËNA. Collegiate Press, Inc., Ames, Iowa, 1938. Pp. xvi+243, figs. 132. Price $3.00.

It has often been said that the soil is in a dynamic state. Mineral and organic compounds in the soil are constantly breaking down. On the other hand, there is a synthesis of new products. Among other factors, various organisms play an important rôle in the formation of new compounds. Until relatively recent times microscopical technique was not adequate for giving us a full insight into the transformations which occur in the soil. The present treatise represents a pioneering effort in opening up new research vistas in soil science.

The author notes in his foreword that:

> As a part of general pedology, micropedology deals with the morphology, genesis, general dynamics and biology of soils. Soil mineralogy, as a counterpart of micropedology, deals with the independent study of the soil minerals, especially the so-called clay minerals. Both branches, though primarily united by the application of microscopic methods, relate to each other as general mineralogy relates to petrography and petrology. Soil mineralogy is, therefore, an ancillary science and not a part of micropedology. There are many unsolved problems in both fields, the solution of which will consume lifetimes of effort.
>
> This book is devoted to the fundamental principles of microscopic pedology. The importance of its application in the different branches of practical soil science, especially in the fields of soil erosion, soil mapping, agricultural soil classification, tillage, engineering and road construction, will be the subject of separate publications to follow.

The contents of the book are arranged in four parts, namely: General; The Technique of Micropedology; Soil Fabrics; and Biological Soil Microscopy. The major topics dealt with in the subdivisions of the book are entitled, respectively: Part I. The Principles of Micropedology; Use and Development of Microtechnique in Other Natural Sciences; Part II. Incident Light Microscopes; The Soil Microscope; Performance of Micromanipulations; Fabric Reactions; Optical Methods; Microchemical Methods; Part III. Introductory; Elementary Fabric; Fabric of Aggregates and Detachment Bodies; Fabric Types in Coherent Soils; Compact Soil Fabrics; Part IV. Characteristics of the Microhabitat; Soil Fabrics and Soil Biology; Observations on Humus Formation; and The Soil Microflora and Fauna Observed by Direct Microscopy.

The student of soils will welcome the new and helpful information which has been provided by the author. The list of references and the subject and author indexes add to the usefulness of the book.

Statistical Methods. Revised Edition. By GEORGE W. SNEDECOR. Collegiate Press, Inc., Ames, Iowa, 1938. Pp. xiii+388, tables 137, figs. 22. Price $3.75.

The author's objective in preparing this book is indicated in the first two paragraphs of the preface. He says:

> The beginner in experimentation too often finds himself supplied with a pair of elaborate mechanisms. In the one hand is a mass of data demanding simplification and interpretation, while in the other is a complex statistical methodology said to be necessary to research. How shall the two be geared together? Since the data can be only inefficiently utilized without statistical method, and since method is futile until applied to data, it seems strange that greater effort has not been made to unite the two. For those of some experience there are adequate texts and journal articles. It is the novice to whose needs this book is directed. It is hoped that he may be furnished with a smoothly working combination of experimental data and statistical method.
>
> Like all other sciences, statistics is in a stage of rapid evolution. During the last 20 years new discoveries have swiftly succeeded each other, fruitful syntheses have been effected, novel modes of thought have developed and a whole series of brand new statistical methods have been marketed. The biologist who has not been able to keep abreast of the progress of statistics finds himself a bit confused by the new ideas and technical terms. It is thought that he will welcome a statement of them in a form that will not require too much distraction of his attention from necessary professional duties.

The body of the book is made up of an introduction, 16 chapters, a general index, and an index of symbols. The chapters are designated as follows: Experiments on Attributes; An Experiment Designed to Compare Measurements of Individuals; Sampling from a Normally Distributed Population; An Experiment Designed to Compare Two Groups; Short Cuts and Approximations; Linear Regression; Correlation; Large Sample Theory; Enumeration Data with Multiple Degrees of Freedom; Experiments Involving More Than Two Groups of Measurement Data. Analysis of Variance; Analysis of Variance with Two Criteria of Classification; Two Variates in Two or More Groups. Covariance; Multiple Regression and Covariance; Curvilinear Regression; Individual Degrees of Freedom; and Large Samples of Enumeration Data. Binomial and Poisson Distributions.

For some decades the student of agricultural science has been gaining more efficient tools for assembling and interpreting statistical data. The present volume is a very desirable addition to the group of reference books on the subject.

Research Methods on Farm Use of Tractors. By N. JASNY. Columbia University Press, New York, 1938. Pp. xxvi+273, tables 18, charts 15. Price $3.75. European Agent: Oxford University Press, London.

This is a most readable book. The editors cannot be accused of exaggeration in having noted in the foreword the far-reaching changes and improvements with which agricultural power machinery may be credited. The following may be quoted from the editors' foreword:

> If the American farmer of a hundred years ago could today travel up and down the broad countryside of the United States, he would be amazed at the transformation. He would

discover not only that the nation's farming area has greatly increased in size but also that the whole process of American agriculture has been revolutionized. Of all the changes which he might note, none perhaps would impress him more than the extent to which agriculture has been almost completely mechanized. Instead of gangs of muscle-hardened, perspiring men, toiling long hours with hoes, forks, scythes, rakes, sickles, cradles, flails, and other primitive tools, he would find sulky and gang plows, mowing machines, hayrakes, tedders, loaders, stackers, grain drills and binders, corn planters and harvesters, threshing combines, milking machines, and mechanical cotton pickers. He would also ascertain that even in those areas devoted to the production of fruit and vegetables mechanical spraying apparatus is considered indispensable.

The effect of these mechanical improvements upon the productivity of agricultural labor has been little short of remarkable. The modern combine, for example, can reap and thresh from 60 to 125 acres of grain a day. If the 1926 Kansas wheat crop had been harvested by the methods in vogue a hundred years ago, 775,000 harvest hands working 20 days would have been needed to cut, bind, and shock the crop. To put it differently, had Kansas been called upon to harvest its wheat crop of that year by hand methods, it would have required all the male population of the state between the ages of 15 and 60 years, and, in addition, all the women of the state between the ages of 20 and 37 years. In a word, without agricultural machinery the agricultural output of the nation since 1850 would have been impossible. The extent to which its use has lightened the burden of farm labor is perhaps incalculable.

The contents of the book include, aside from the editors' foreword, acknowledgements, introduction, and 10 chapters, designated as follows: Types of Tractors; Size of Tractors; Grouping of Farms according to the Type of Power; Methods of Computing the Cost of Operating Tractors; The Procedures Followed in Power Investigations; The Basis for Comparisons of the Costs of Farm Power; Adjustments of the Cost of Power; The Effect of Farm Size on the Cost of Power; Selecting the Area; and Summary. There are also four appendixes, named: Rated and Adjusted Horsepower of Tractors; The Recommended Method of Computing the Rate for Depreciation of Tractors, with Comparisons; Some Details of the Application of the Recommended Method of Computing the Charge to Depreciation; and Derivation of Depreciation Formulae. A bibliography and an index complete the material included in the book.

This book should attract interest among economists, agricultural engineers, agronomists, and students of soils. Students in vocational agricultural classes, undergraduates and graduate students, as well as extension workers and progressive farmers, will find this work a desirable addition to their reference shelves.

Ferns of the Southeastern States. By JOHN KUNKLE SMALL. The Science Press, Lancaster, Pa., 1938. Pp. 517, illus. Price $3.50.

One of our noted educators, despite his fourscore years, is still searching for the last palm not yet described. His tireless search for new species is characteristic of the lover of plants. Ferns, like other plants, have attracted the attention of botanists. There are many of them, and they are of great interest because of their past as well as of their present history and appear-

ance. At least one paragraph should be quoted by the reviewer from the author's preface. He says:

> Up to the beginning of the present century fern collecting and studies in Florida were largely incidental to travelling botanists or to special attention in quite circumscribed areas. In the first decade of this century the hidden fern treasures in hammocks of the Pleistocene Everglade Keys and vicinity at the southern end of the Florida peninsula were tapped by the writer and his associates in exploration. This region was up to that time quite difficult of access. Beginning with the third decade the last region to be thoroughly explored for ferns—the ancient tropical Oligocene Island region in the upper part of the peninsula—was made the object of an intensive fern study by Edward P. St. John and Robert P. St. John. The results of these remarkable explorations and studies are recorded on the following pages, especially under the genera *Trichomanes*, *Thelypteris*, and *Ophioglossum*.

The contents of the book include: Preface; Introduction; Map; Key to the Orders, Families and Genera Illustrated for Students; Taxonomic Treatment, with Distribution and Notes; General and Restricted Distribution of the Species; on the Cultivation of Native Southeastern Ferns (W. A. Knight); Taxonomic List with Citations (J. H. Barnhart); Authorities Cited in this Work (J. H. Barnhart); Glossary; and Index.

The author has succeeded in arranging the material in a manner to make it most helpful to the reader. Particular credit should be given to the author and the publishers for the excellent illustrations. This book is valuable as well as interesting.

The Macaulay Institute for Soil Research. Collected Papers. Vol. 1. Edited by W. G. Ogg. The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, Scotland, 1938. Price 21/–.

General science has gained much from the specialized studies in the field of soil science. New information in the latter field is being contributed constantly by able students in Europe, North America, and elsewhere. *The Collected Papers* constitute a distinctive and satisfactory contribution from the Macaulay Institute.

It may not be amiss here to quote a portion of the preface by Sir Robert Greig. It is to be regretted that lack of space will not allow the quoting of the entire preface. The following may be noted:

> In 1930 the Macaulay Institute was incorporated and a Committee of Management appointed. The history of the Institute since then is to be found in the contents of this volume. Under the Director the work of the Institute has steadily increased; this has meant additions to the staff and buildings, while the value of the work of the Institute to farmers and to science in general becomes more evident every year. Many different lines of soil work are undertaken at the Institute, one of the most important being investigations on general soil fertility and advisory work amongst farmers on problems of liming, manuring and soil management. In accordance with the intention of the founder, a special study is made of the poorer classes of land in Scotland, including peatland, the soil survey work is carried out with a view to mapping the soils of the country by the most up-to-date methods of field study and of chemical and geological laboratory examination. The study of soil

drainage which was previously carried on by the North of Scotland College of Agriculture, under the direction of Professor Hendrick, has been continued at the Institute.

The major topics dealt with in this book are: Foundation of Institute; Chemical and Physical Studies; Methods of Analysis; Peat Reclamation and Land Improvement; Soil Fertility; Soil Surveys and Classification; and Miscellaneous. There are 54 contributions making up *Collected Papers*. They cover a wide variety of topics and possess more than ordinary interest and value for the student of soils.

Rothamsted Experimental Station Report for 1937. Gibbs & Bamforth, Ltd., St. Albans, England. Pp. 225.

Earlier reports from the Rothamsted Station have been reviewed in this journal. The present report will bring up to date the extremely valuable data that the station has been accumulating for nearly a century.

The contents of the report are well described in a brief statement furnished by the director of the station. This statement follows:

Farmers and their technical advisers will find a great deal of useful information on crop production and the use of fertilizers in the present issue of the Rothamsted Report covering the activities of the Station during 1937.

The volume contains the full data relating to the numerous field experiments carried out at Rothamsted and many outside centers, and also valuable summaries of groups of experiments dealing with special subjects. Although farmyard manure is by far our most important soil improver, its effects have not been measured nearly so frequently as those of artificials. All the available experiments with dung have been collected in the present report. They bring out its direct effect on potatoes, sugar beet and kale and its residual action on the following crops of cereals. An important point illustrated is the effect of the presence of dung on the action of artificial fertilizers. A further section sets out the main results of 107 fertilizer experiments on sugar beet carried out during the past four seasons on a uniform plan in all the important beet growing districts. Salt, one of the oldest of manures, has recently been carefully studied in its effects on sugar beet and mangolds. Its action has usually been very favorable on these crops and evidence on this point is collected from a number of centers. Under the present regulations for the sale of potatoes the size of the tubers is of considerable importance, and a review of the effects of manures on the proportion of ware potatoes is given. Liming experiments are also reported which show that in certain soils the effect of even a light application of chalk can persist throughout a long rotation.

A full account of the laboratory work is given, supplemented by abstracts of recent papers. In the Chemical Department the numerous field experiments have provided material for the study of analytical methods for estimating the manurial requirements of soils, and particular attention is being devoted to rapid methods of soil analysis. Perhaps one of the most interesting aspects of the biological work is the study of strains of the nodule organisms of leguminous plants. Some of these strains instead of being beneficial have been shown to be actually parasitic and in addition they are so aggressive that they can displace many of the strains that show the usual beneficial action on the plant. Fortunately certain strains have been discovered that are both beneficial and also capable of competing with the worthless strains.

The report contains a summary of twenty years' work in the Department of Plant Pathology in which the contributions relating to the study of Wart Disease of Potatoes, Take-all disease of Wheat and the group of Virus diseases are set out with full references.

The reader of the report is certain to be impressed by the wide range of scientific investigations conducted at the Rothamsted Experimental Station and by the scientific value of the data presented.

Commercial Fertilizers. Second Edition. By GILBEART H. COLLINGS. P. Blakiston's Son & Co., Inc., Philadelphia, 1938. Pp. xvii+456, figs. 109, tables 88. Price $4.00.

This is the second edition of a very useful book. Much new material had become available since the printing of the first edition, as is indicated by the author. He says:

> Within the brief period of four years, since the appearance of the first edition of this book, the numerous contributions to our knowledge of the rapidly growing subjects of soil fertility, crop nutrition, and fertilizer manufacture have brought about a necessity for changes in the new edition. The book has been brought to date by the incorporation of much additional information. Some chapters have been almost entirely re-written and many new paragraphs have been added to others. One new chapter has been added which deals with the problems of adjusting soil reaction and fertilizer practice to crop requirement.
>
> In this edition it has been found necessary to greatly enlarge the bibliography although every effort has been made to hold the number of references to a minimum.
>
> It is hoped that the book may continue to be useful as a college text, as a reference book for those interested in fertilizer manufacture, and as a source of information for everyone interested in growing larger yields of field and horticultural crops.

The book contains 16 chapters, designated, respectively: Origin and Development of the Use of Commercial Fertilizers; Source, Production and Use of Sodium Nitrate; Manufacture and Use of Ammonium Sulphate; Manufacture and Use of the Synthetic Nitrogenous Fertilizers; Sources and Uses of Organic Nitrogenous Fertilizers; Sources and Use of the Mineral Phosphates; Sources and Use of Bone Phosphate and Basic Slag; Manufacture and Use of the Superphosphates; Production, Manufacture and Use of the German and French Potash Salts; American Sources of Potash Fertilizers; Source and Use of Fertilizers Carrying Essential Elements Other Than Nitrogen, Phosphorus and Potassium; Fertilizers Carrying Elements Not Accepted as Essential for Plant Growth; Principles Underlying the Purchase of Fertilizers; Principles Underlying the Use of Fertilizers; The Application of Fertilizers, and the Influence of Fertilizers on Germination and Seedling Growth; and Adjusting Soil Reaction and Fertilizer Practice to Crop Requirement. There are also a bibliography, an authors' index, and a general index.

The author has been able to collect, interpret, and present to the reader in useful form the recent developments in the use of commercial fertilizers. He has dealt with them in their relation to soils and likewise to growing plants. For the teacher and student the second edition of *Commercial Fertilizers* will prove to be very useful and interesting.

The Chemistry of the Amino Acids and Proteins. Edited by CARL L. A. SCHMIDT. Charles C. Thomas, Springfield, Ill., and Baltimore, Md., 1938. Pp. xxiv+1031, tables and figs. Price $7.50.

The editor of this book has been able to enlist the services of a substantial group of able and well-informed contributors. They have reviewed their respective fields in a way to have placed many students and teachers under obligation to them. The editor notes in his preface that:

> During the past twenty years information regarding the properties and the behavior of the amino acids and proteins has not only expanded considerably, but has also increased in exactitude. Extensive investigations have been directed to the study of the physico-chemical and thermodynamic properties of amino acids and proteins. This information has done much towards promoting a better understanding of these substances in life processes.
>
> The hypothesis of the zwitterion structure of amino acids is now so generally accepted that it is no longer regarded as a theory. Better methods for isolating and synthesizing amino acids and peptides have been devised. Nutritional studies have not only shown the indispensability of certain amino acids, but have also led to the discovery of hitherto unrecognized ones. Important progress relative to the metabolism of the amino acids in the animal organism has been made. The coöperative efforts of biochemists and immunologists have brought out the importance of the chemical constitution of proteins to immunological specificity.

Part I of the book contains 10 chapters, designated respectively: Historical; The Constitution and Synthesis of the Amino Acids; The Isolation of the Amino Acids from Proteins. The Preparation of Amino Acids and Proteins; Methods of Analysis and Reactions of the Amino Acids and Proteins; Relation of the Amino Acids to Products of Biochemical Importance; Peptides, Peptidases, and Diketopiperazines; The Chemical Constitution of the Proteins; Molecular Weights of the Proteins; Certain Chemical and Physical Characteristics of the Proteins; and Optical Properties of Amino Acids and Proteins. Part II contains 8 chapters, designated, respectively: Amphoteric Properties of Amino Acids and Proteins; Electrochemistry of Amino Acids and Proteins; Combination of Amino Acids and Proteins with Acids, Bases, Heavy Metals, and other Compounds; Membrane Equilibria; Some Thermodynamical Considerations of Amino Acids, Peptides, and Related Substances; Dipolar Ionic Structure and Solubility of Amino Acids, Peptides, and Proteins; Relation of Proteins to Immunity; and The Rôle of Proteins in Nutrition. There are also an appendix and author and subject indexes.

In a sense, this is a monumental work, and certainly an extremely valuable addition to our reference works on the chemistry of the amino acids and proteins. The student of organic chemistry and the biochemist should not fail to place a copy of this book on his reference shelf.

Algae. The Grass of Many Waters. By LEWIS HANFORD TIFFANY. Charles C. Thomas, Springfield, Ill., and Baltimore, Md., 1938. Pp. xiii+171, Frontispiece, figs. 12, plates 41. Price $3.50.

The subtitle of this book is very suggestive. Even more suggestive is the preface. At least one paragraph from it should be quoted. The author tells that:

> If the author of this book were a fish or a frog, he could doubtless write a very entertaining account of the algae: at least it would be different. That a fish would describe an alga as a

"low form of plant able to manufacture its own food" is quite uncertain indeed. Whether or not a frog would approve of such epithets as "pond scums," "frog spit," "that green moss," or "plant debris" is very much unknown. Really, I suspect, a fish—a little fish anyway—generally regards many of the algae as great stationary or oscillating forests of intertwining, variously colored, slippery, slimy strands through which it must swim in hunting for its breakfast or in dodging another hungry fish. At some time during its life, however, the average fish must regard algae as a rabbit considers lettuce, as a horse enjoys green pastures, or as Rastus thinks of watermelons: in other words, as something to eat.

Aside from the preface and acknowledgements, we find 13 chapters, a list of general references, and an index. The chapters are designated, respectively: What Are Algae?; Algae and the Foods They Make; How Algae Grow and Reproduce; Algae of Lakes and Ponds; Algae of Streams and Rivers; Algae of the Sea; Algae of the Soil; Algae of Ice and Snow; Algae of Bizarre Abodes; Algae of the Past; Algae and Human Welfare; How to Collect Algae; and How to Study Algae.

This is a most readable and entertaining book. The illustrations are good and are an aid to visualizing to the reader the intricate world of algae.

The Principles of Soil Science. By ALEXIUS A. J. DE'SIGMOND. Thomas Murby & Co., London, 1938. Pp. xiv+362, figs. 34, tables 105, plates 4.

This book was translated from the Hungarian by Arthur B. Yolland, the translation edited by G. V. Jacks, and a foreword prepared by Sir John Russell. The author—a distinguished student of soils and one of the consulting editors of this journal—has made many important discoveries in the field of soil chemistry.

In his foreword to the book, Sir John Russell says:

The main theme of the book (published some years ago in longer form in Hungarian) is the presentation and discussion of the system of soil classification already known internationally by the author's name, but never before described in detail in an English publication, consequently it has not received from English and American investigators the careful scrutiny which it merits as a positive contribution towards the advancement of soil science. No harm can result if some readers find themselves in disagreement with the author on making first acquaintance with the details of his system. Every country possesses certain soil types not found elsewhere; certain English soils, for example, do not easily fit into Professor de Sigmond's system, and certain Hungarian soils fit into no English system. The comprehensive classification here proposed, based essentially on the chemical composition of the soil, is not intended to be final, but it satisfactorily fills many gaps in other systems, and leads us a considerable step further towards the goal of every soil taxonomist—a universal classification based on strictly scientific principles.

Aside from the foreword, the author's preface, and the introduction, the reader will find in the book, 18 chapters, and subject and author indexes. The four parts of the book are entitled, respectively: Genetics; Agronomy; Soil Systematics; and Principles of Soil Cartography. The chapters themselves are designated, respectively: Geological and Petrographic Soil-Forming Factors; Climatic Soil-Forming Factors; Orographical (local) and Hydro-

graphical Conditions as Soil-Forming Factors; Natural Vegetation as a Soil-Forming Factor; Animals as Soil-Forming Factors; Micro-organisms as Soil-Forming Factors; The Age of Soils. Time as a Soil-Forming Factor; Man as a Soil-Forming Factor; The Principal Soil-Forming Reactions; Local Soil Surveys; Chemical Properties of Soils and Their Characterisation; Introduction; The General Soil System; Characterisation and Further Classification of Soil Types (Stages IV, V and VI, of the Soil System); Physical and Physiological Classification of Local Varieties (Stages VII and VIII); Various Types of Soil Maps; Laws Governing the Geographical Distribution of Soil Types; and To What Extent Does the Actual Distribution of Soil Types Agree with the General Soil System?

The American student of soils will feel grateful to the translator for having made available to him material not otherwise accessible. It will be found to be a useful book for teaching and reference purposes. Beyond that, it may be noted that the American student of soils will find his horizon widened and his terminology amplified through his becoming better acquainted with soil regions in the Old World.

Genetics. An Introduction to the Study of Heredity. Fourth Edition. By HERBERT EUGENE WALTER. The Macmillan Company, New York, 1938. Pp. xvii+412, figs. 150. Price $3.00.

This is the fourth edition of a book which is well and favorably known. The author, who is Emeritus Professor of Biology at Brown University, has a keen appreciation of the significance of genetics. He notes in his preface that:

> The great-grandfather of this book, the first in the ancestral line to bear the name *Genetics*, saw the light of day in 1913, and was the direct outcome of some years of inspiring association with Professor William E. Castle of Harvard University, and Dr. Charles B. Davenport of the Carnegie Institution of Washington at Cold Spring Harbor, who were two of the earliest pioneers in America in modern studies of heredity.
>
> The grandfather of the series, bearing the qualifying title of "*Revised Edition*," was born in 1922, and the father, of "*Third Edition*," came along in 1930, just in time to participate in the Great Depression.
>
> Now in 1938 appears the fourth in line, a somewhat different young hopeful, still bearing the family name but looking back upon its underprivileged ancestors with something of the apologetic tolerance of youth.

The book includes a preface and 11 chapters, designated, respectively: Introduction; The Observational Avenue of Approach; The Experimental Method of Approach; The Statistical Approach; The Cytological Method of Approach; The Architecture of the Germplasm; The Contributions of Sex; The Developmental Method of Approach; The Application to Man; Human Conservation; and Problems for Practice. There are also 9 appendixes, named: Statistical Mill for Measuring Variation; Trihybrid Blocks; Tracing the Family Distribution of a Single Trait; A List of Possible Human Traits,

Hereditary or Acquired; Suggested Topics for Eugenics Theses; Sample Correct-Incorrect Test; Useful Addresses; Contacts of Genetic Interest Outside the Class Room; and The Survey of Human Resources of Connecticut, 1937. The appendices are followed by a bibliography and an index.

It is obvious that, in preparing the fourth edition, the author has lost nothing of his skill and his ability to express himself clearly and effectively. Many teachers and students will recognize their debt to the author and will grant that he has been able to instruct them and to entertain them as well.

JACOB G. LIPMAN

GIACOMO ROSSI

Giacomo Rossi

1872-1938

The long career of Professor Giacomo Rossi, who was active for many years in several fields of microbiology, was terminated by death on May 1, 1938, at the age of 65 years. He succumbed in a hospital in Naples after having been confined by serious illness for some weeks.

He was born in Reggio Emilia, Italy, on October 15, 1872, received his degree in medicine at Rome in 1897 and, two years later, a degree in chemistry, at the University of Modena. His first position was that of assistant in general pathology and pathological anatomy in Modena. Later, he became affiliated with the Institute of Hygiene at the University of Naples, and, finally in 1902, joined the department of agricultural bacteriology at the Royal Agricultural Institute of Portici. In 1904 he became Professor of Agriculture and Technical Microbiology in this institute, which later became affiliated with the Royal University of Naples.

Though Prof. Rossi is better known outside Italy and especially to soil scientists for his contributions to soil microbiology, his interests extended far beyond this field into the realms of rural hygiene and of medical and industrial microbiology. Much of his attention was devoted to studies of malaria, its control and relationships to the development of agriculture in Italy; the processes of pectin decomposition and the retting of fibers of textile plants were also investigated in considerable detail; additional subjects of study in the industrial field included the fermentation of tobacco, alcoholic fermentation, and the bacteriology of milk and cheese. Among his reports are publications on silage, plant diseases, and forestry.

His contributions to soil microbiology include a new method of studying the soil population as well as studies of the development of microörganisms in the soil material originating from lava of Mt. Vesuvius. This particular method has attracted unusual attention, by reason of its simplicity and effectiveness, and has proved extremely valuable in the hands of many investigators who, through its use, have been able to add to the knowledge of the aggregation of microbial cells in the soil and the influences of various soil materials and environmental conditions upon the localization of the bacterial cells. The method has commonly been called the "contact slide method" or the "Rossi-Cholodny method"; this latter designation was applied in view of the fact that, although Cholodny's observations followed those of Rossi, they considerably extended the scope and significance of the latter's results and were the original source of information to many readers.

The procedure as first used was to press a clean microscope slide against a

soil surface; remove it; dry, fix, and stain the adhering film; and examine the stained material microscopically. The preparations gave good reproductions of the localization of the microörganisms and the manner of distribution characteristic of their development in the soil environment. The method was later modified to leave slides buried in the soil for various periods of time. By this means, the soil organisms which developed over the glass surface could be seen in their typical arrangements after the slides were removed and stained.

In addition to his research activities, Professor Rossi took a prominent part in various agricultural affairs. Of these, particular mention should be made of the fact that he served as consulting editor of SOIL SCIENCE during the entire 22 years of its publication and was editor of *Annali di Tecnica Agraria*, an Italian journal devoted to original papers and abstracts in agricultural science, for the 11 years that it has been issued. He also served on numerous agricultural commissions in his own country.

The contributions of Professor Rossi to soil science have been numerous and should encourage and stimulate subsequent investigators.

ROBERT L. STARKEY

COMPARATIVE STUDY OF THE COLLOIDS OF A CECIL AND A SUSQUEHANNA SOIL PROFILE

W. P. KELLEY, A. O. WOODFORD,[1] W. H. DORE, AND S. M. BROWN

University of California

Received for publication August 27, 1938

In connection with investigations on the colloidal materials of California soils it became desirable to make a corresponding study on soils which have been subjected to relatively heavy leaching. Profiles of the Cecil and Susquehanna series were chosen for this purpose. The Cecil, as is well known, is a residual soil derived from schistose granite-gneiss. It occurs in the southern part of the Piedmont Plateau where the rainfall is comparatively heavy. The Susquehanna is a sedimentary soil of marine origin found at various places in Alabama, Mississippi, and other southern states.

DESCRIPTION OF THE PROFILES

The Cecil samples were taken[2] from a deep, recently formed gully, 5 miles north of Auburn, Alabama, where the entire profile is exposed down to and including the slightly altered schistose granite gneiss rock.

18830 (0–6 inches) A horizon, gray sandy soil comparatively low in clay.
18831 (8–15 inches) B_1 horizon, red clay.
18832 (At 6 feet) B_2 horizon, showing some evidence of illuviation.
18833 (At 12 feet) C horizon, decomposed granite-gneiss without evidence of illuvial enrichment.
18834 (At 20 feet) decomposed parent material near the upper limits of granite-gneiss.
18835 (At 24 feet) slightly decomposed granite-gneiss, sometimes referred to as "granite sand."

The Susquehanna samples were taken[3] from a road cut in the Union Springs-Montgomery Highway, about 23 miles northwest of Union Springs, Alabama, and comprised the profile which Baver and Scarseth (2) discussed in 1931. The location is about 60 miles southwest of the place where the Cecil samples were taken.

16312 (0–8 inches) A horizon, grayish brown loamy fine sand.
16313 (8–20 inches) B_1 horizon, bright red clay.
17554 (20–30 inches) B_2 horizon, sticky, heavy red clay with yellowish mottling.
17555 (At 10 feet) C horizon, blue sandy clay slightly mottled.
17556 (At 11 feet) grayish white clay layer about 10 inches thick. Material similar to that at 10 feet occurs below this layer.

---

[1] Professor of geology and mineralogy, Pomona College, Claremont, California.
[2] By G. D. Scarseth, formerly of the Alabama Agricultural Experiment Station.
[3] By J. W. Tidmore, of the Alabama Agricultural Experiment Station.

A portion of the 1-μ colloidal material was separated from the several horizons of each profile by vigorously shaking several hundred grams of the original samples with distilled water and then siphoning off the top 8 cm. after standing for 24 hours. The colloidal materials held in suspension in the siphonate were coagulated by adding calcium chloride, after which the solutions were filtered, and the colloids were then leached with normal calcium acetate solution until the base-exchange materials were approximately calcium saturated. The excess of calcium acetate was removed by leaching with methyl alcohol, and the organic substances were decomposed by H_2O_2 oxidation on a water bath. Following the treatment with H_2O_2, the colloids were again leached with calcium acetate solution, then with methyl alcohol, and finally they were dried at laboratory temperature. The materials thus obtained were therefore calcium saturated and approximately free from organic

TABLE 1

*Mechanical analysis of the Cecil profile**

SAMPLE NUMBER	DEPTH	FINE GRAVEL (2–1 MM.)	COARSE SAND (1–0.5 MM.)	MEDIUM SAND (0.5–0.2 MM.)	FINE SAND (0.2–0.1 MM.)	VERY FINE SAND (0.1–0.02 MM.)	SILT (0.02–0.002 MM.)	CLAY (<0.002 MM.)	FINE CLAY (<0.001 MM.)	pH
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
18830	0–6	4.3	13.3	10.3	23.9	18.4	16.1	13.8	10.5	5.5
18831	8–15	7.0	14.6	7.9	13.4	11.2	11.0	34.8	31.5	5.1
18832	72	8.2	17.6	8.5	12.6	11.7	11.4	30.9	27.8	5.1
18833	144	7.4	15.1	8.3	18.7	15.8	7.3	28.2	26.7	4.8
18834	240	7.2	22.6	13.8	27.8	17.8	7.0	4.2	3.6	5.2
18835	288	4.9	24.9	15.2	33.4	15.9	3.1	2.3	2.6	5.5

* Analyses by Charles R. Horton.

matter. Extensive experience has shown that this method of preparation produces no important change in the clay minerals of soil colloids.

The Cecil profile

Table 1 shows the mechanical analysis and pH of the Cecil profile.[4] It will be noted that the samples taken at depths of 8–15 inches, 6 feet, and 12 feet, respectively, contain substantially more clay than does the surface soil, the decomposed parent material at 20 feet in depth, or the slightly altered granite-gneiss. From the standpoint of the colloidal materials the most important point brought out by these mechanical analyses is the fact that 1-μ clay constitutes by far the greater part of the total clay of these samples. It will be noted that this profile is strongly acidic at all depths sampled, including the slightly altered granite-gneiss.

Table 2 gives the analysis of the 1-μ calcium-saturated Cecil colloids. The

[4] The mechanical analysis of both the Cecil and the Susquehanna samples was made under the direction of C. F. Shaw by Charles R. Horton, using the U. S. Bureau of Soils method.

data show remarkable uniformity in composition of colloid throughout the profile, the only exception being found in the sample taken at a depth of 12 feet. Here the colloid was somewhat richer in SiO_2 and poorer in Fe_2O_3 than that of the horizons either above or below this depth.

Table 3 shows the molecular ratios and base-exchange capacities of these colloids. The base-exchange capacity, being determined by the ammonium acetate method, gives a measure of the power of the colloid to absorb NH_4 from a neutral, normal solution of ammonium acetate. These data show that the colloid as separated was remarkably uniform throughout this profile. The silica-alumina ratio is only slightly less than 2 in the samples from all the

TABLE 2

Chemical analysis of Ca-saturated Cecil colloids

SAMPLE NUMBER	DEPTH	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	P_2O_5	CaO	MgO	K_2O	Na_2O	H_2O	TOTAL
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
18830	0–6	36.49	33.57	8.02	1.00	0.03	0.26	0.62	0.54	0.90	0.23	18.87	100.53
18831	8–15	36.57	34.76	8.78	1.00	0.08	0.23	0.47	0.54	0.47	0.36	17.06	100.32
18832	72	37.94	34.97	8.14	1.10	0.10	0.16	0.44	0.42	0.52	0.41	16.21	100.41
18833	144	42.49	34.29	4.76	1.00	0.09	0.31	0.44	0.28	0.96	0.31	16.00	100.93
18834	240	38.87	34.24	8.70	0.80	0.09	0.33	0.34	0.29	0.40	0.36	15.25	99.67

TABLE 3

Molecular ratios and base-exchange capacities of Cecil colloids

SAMPLE NUMBER	DEPTH	$\frac{SiO_2}{Al_2O_3}$	$\frac{SiO_2}{R_2O_3}$	BASE-EXCHANGE CAPACITY
	inches			*m.e.*
18830	0–6	1.84	1.60	22.0
18831	8–15	1.79	1.54	17.5
18832	72	1.84	1.60	16.5
18833	144	2.13	1.94	16.3
18834	240	1.93	1.66	13.0

horizons except that at 12 feet, where it slightly exceeds 2. The base-exchange capacity is also fairly constant and low in each case. These data are consistent with the conclusion that the colloidal material throughout this profile is composed primarily of either kaolinite or halloysite.

The Susquehanna profile

Table 4 reports the mechanical analysis and pH of the Susquehanna profile. The results show that the A horizon contains very much less clay and correspondingly more fine sand than the samples drawn at various depths below this horizon. As in the case of the Cecil profile, 1-μ clay constitutes by far the greater proportion of the total clay.

The fact that the entire profile down to and including the light-colored clay layer at 11 feet in depth is strongly acidic, is worthy of special notice. When the materials were laid down in sea water it is virtually certain that they were alkaline, because ocean water usually has a pH of about 7.8 to 8.0. Moreover, only traces of chlorine were found in any of the samples. Apparently leaching has been sufficiently intense not only to remove soluble salines from the entire profile, but also to bring about the replacement of significant amounts of exchangeable bases by hydrogen ions. These results agree very well with those reported by Baver and Scarseth (3).

TABLE 4

*Mechanical analysis of the Susquehanna profile**

SAMPLE NUMBER	DEPTH	FINE GRAVEL (2–1 MM.)	COARSE SAND (1–0.5 MM.)	MEDIUM SAND (0.5–0.2 MM.)	FINE SAND (0.2–0.1 MM.)	VERY FINE SAND (0.1–0.02 MM.)	SILT (0.02–0.002 MM.)	CLAY (<0.002 MM.)	FINE CLAY (<0.001 MM.)	pH
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
16312	0–8	0.1	0.2	0.4	45.1	45.2	5.0	4.5	3.7	5.2
16313	8–20	0	0.1	0.2	18.6	19.6	7.7	53.6	52.8	4.0
17554	20–30	0.1	0.2	0.4	35.8	16.0	5.0	42.1	38.3	4.4
17555	120	0.1	0.3	0.4	30.7	22.3	6.4	40.1	37.5	4.4

* Analyses by Charles R. Horton.

TABLE 5

Chemical analysis of Ca-saturated Susquehanna colloids

SAMPLE NUMBER	DEPTH	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	P_2O_5	CaO	MgO	K_2O	Na_2O	H_2O	TOTAL
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
16312	0–8	44.69	27.91	6.34	0.54	0.05	0.35	0.80	1.33	0.34	0.37	17.60	100.32
16313	8–20	41.09	29.93	8.84	0.36	0.07	0.43	0.95	1.17	0.24	0.33	16.93	100.34
17554	20–30	44.89	26.14	7.84	0.56	Tr.	0.15	1.68	1.65	0.46	0.39	17.80	101.56
17555	120	47.95	24.01	5.64	0.33	Tr.	0.32	1.76	2.31	0.50	0.43	19.00	102.25
17556	132	52.21	22.41	3.26	0.25	Tr.	0.16	2.55	2.75	0.26	0.37	14.30	98.52

In contrast to the Cecil, the Susquehanna colloid from the A horizon proved to be somewhat richer in silica than that of the B_1 horizon, whereas the colloids of the B_2 and C horizons and the light-colored clay layer at 11 feet in depth contained silica in amounts increasing with depth (table 5). The opposite was found as regards the sesquioxides. Both CaO and MgO increased with depth. It should be borne in mind that these colloids were all artificially Ca-saturated, and it is of interest to note that the major portion of the CaO found was present as exchangeable calcium. It should also be pointed out that the exchangeable calcium was almost exactly equal quantitatively to the NH_4-absorbing power of these colloids. This shows that the samples were really Ca-saturated, which, of course, resulted from the treatment with cal-

cium salt solutions. On the other hand, scarcely any of the MgO in the samples, as analyzed, was exchangeable. The colloid of the A horizon probably contained a greater amount of quartz than the other horizons, which would account for the higher silica and somewhat lower sesquioxide content than in the B_1 horizon. On the other hand, the pronounced changes in composition of the colloids in passing to greater depth, especially as regards silica, sesquioxides, and magnesium, indicate the presence of variable amounts of different clay minerals. The same is indicated by the molecular ratios and the base-exchange capacities (table 6). Both of these values increased markedly in passing downward from the B_1 horizon. These results also agree with those of Baver and Scarseth (3). The colloid of the light-colored layer at 11 feet in depth had a SiO_2/Al_2O_3 ratio of approximately 4 and an extraordinarily high

TABLE 6

Molecular ratios and base-exchange capacities of Susquehanna colloids

SAMPLE NUMBER	DEPTH	$\frac{SiO_2}{Al_2O_3}$	$\frac{SiO_2}{R_2O_3}$	BASE-EXCHANGE CAPACITY
	inches			*m.e.*
16312	0–8	2.73	2.38	30.5
16313	8–20	2.34	1.97	38.4
17554	20–30	2.92	2.45	59.6
17555	120	3.40	2.96	64.7
17556	132	3.95	3.64	95.6

base-exchange capacity of 95.6 m.e. per 100 gm. As will be shown later, the clay of this layer is composed of almost pure montmorillonite.

DEHYDRATION OF THE COLLOIDS

Kelley, Jenny, and Brown (12) showed that certain soil colloids can be differentiated on the basis of their hydration curves. Accordingly, a similar study was made on the colloids of the Cecil and Susquehanna profiles. Before dehydration, the samples were brought to comparable moisture conditions by exposure to an atmosphere of constant water content at 25°C. until they came to constant weight. The samples were then heated to constant weight at 50° intervals throughout the temperature range from 100° to 800°C. The results are illustrated in figures 1 and 2.

The colloid from each horizon of the Cecil gave remarkably similar dehydration curves. These curves agree well with that found on a different sample of Cecil colloid, as reported by Kelley, Jenny, and Brown (12). Instead of smooth dehydration curves of the adsorption type, these curves show pronounced deflections. The first deflection was at a temperature of about 400°C., and the second at approximately 450°C. The shape of these curves is similar to that of finely ground kaolinite or halloysite. The greater part of the

OH ions of the lattice of kaolinite and halloysite pass off as water vapor between 400° and 450°C.

The colloid from the several horizons of the Susquehanna profile showed an interesting variation, which was previously pointed out by Kelley, Jenny, and Brown (12). The colloid of the A horizon gave a dehydration curve very similar to the curve for the Cecil colloids, which, as stated already, resembles

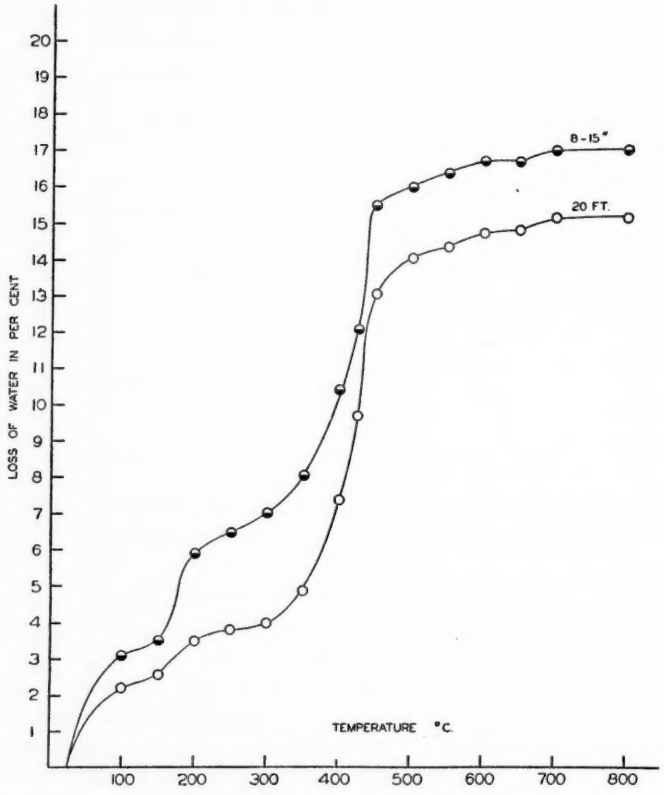


FIG. 1. DEHYDRATION CURVES OF CECIL COLLOIDS
Graphs for the other Cecil horizons were similar to those shown

kaolinite or halloysite. On the other hand, the light-colored clay, found at a depth of 11 feet, gave a dehydration curve which closely approaches the curve for montmorillonite. The dehydration curves for the colloids of the B and C horizons of the Susquehanna were intermediate between those of the surface and the 11-foot horizons. They are suggestive of the curve for beidellite, as shown by Kelley, Jenny, and Brown.

The dehydration curves of the Cecil colloids indicate that throughout the profile the colloidal material is composed largely of one single type of kaolinite-halloysite-like clay, whereas the dehydration curves of the Susquehanna colloids indicate a preponderance of the kaolinitic type of clay in the super-

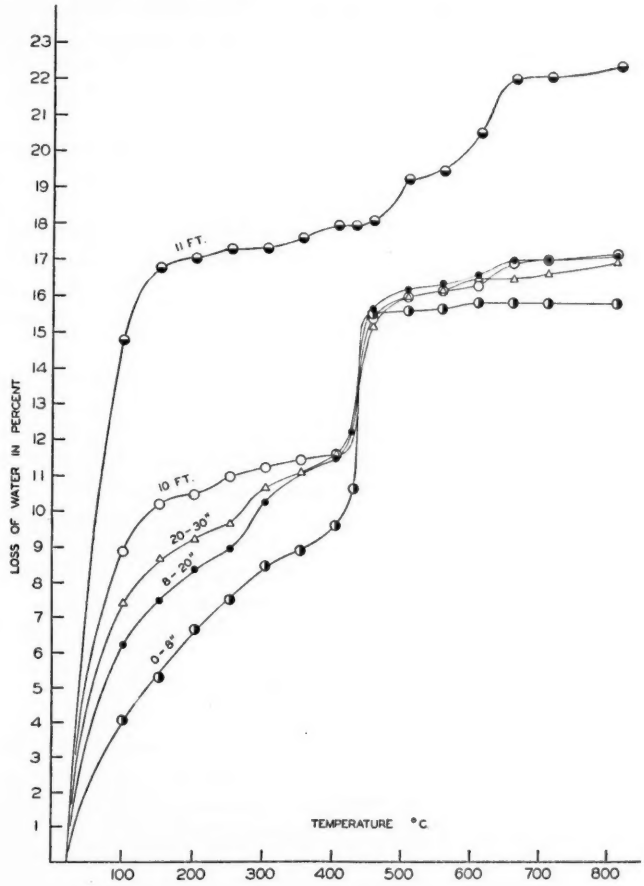


FIG. 2. DEHYDRATION CURVES OF SUSQUEHANNA COLLOIDS

ficial horizons and of beidellite or montmorillonite in the deeper horizons. These data are fairly consistent with the chemical analyses and lead to the same general conclusions. As will be shown in the following sections of this paper, they are also in harmony with the optical measurements and the X-ray data.

OPTICAL STUDIES

Cecil colloids

The Cecil colloids are characterized by: their color, which ranges from deep red-brown to chocolate-brown; high indexes of refraction; and low double refraction or isotropic character (table 7). The colloids from the different horizons of this profile are very similar in optical properties. Perhaps their greatest difference, although not very pronounced, is in color, which ranges from chocolate-brown in the A horizon to deep red-brown in the B and C horizons.

The fairly fresh rock found at a depth of 24 feet is a coarsely foliated biotite-granite-gneiss. The light brown colloid (18834), separated from the samples taken at a depth of 20 feet, includes about 20 per cent biotite shreds in various stages of decay. The biotite flakes, observed in the Ca-saturated, air-dried colloid, were from 10 to 40 μ in diameter. Some of these were sufficiently fresh to show characteristic pleochroism, but most of them have lost this property and were pale brown in color with low birefringence, probably not over .010.

The Cecil colloid 18833 from a depth of 12 feet is dark red in color and more uniform in composition than 18834. No altered biotite was definitely recognized in this sample, although it contained a few plates with double refraction up to .010, which were probably altered mica. By far the greater part of this sample was made up of nearly isotropic, red-brown flakes, composed of minute particles. Most of these particles were yellow and apparently gelatinous, but some of them were translucent and dark red (iron oxide). The colloids from the A and B horizons contain somewhat greater amounts of doubly refracting grains, but their birefringence was very low.

The optical properties suggest that throughout this Cecil profile the colloid is composed chiefly of halloysite and iron oxide.

Susquehanna colloids

The Susquehanna colloids are very different optically from the Cecil colloids. Their high double refraction stands in marked contrast to the low birefringence of the Cecil. For corresponding horizons, the refractive indexes are distinctly lower than those in the Cecil samples. The colloid of the A horizon (16312) was made up of brown flakes and irregular aggregates of minute grains. The flakes showed moderate double refraction and rather high indexes, the latter perhaps being influenced by the iron content. The B_1 colloid (16313) is deep brown in color with refractive indexes slightly higher than those of the A horizon. These two properties correlate well with an iron content higher than that of the A horizon, but the higher birefringence must have a different explanation. The B_2 colloid (17554) has lower indexes and is paler than the B_1 colloid (16313) but is still fairly deep brown. Its double refraction (.020) is as high as that of montmorillonite. The colloid from a depth of 10 feet (17555) was pale

TABLE 7

Optical properties of the colloids

SERIES	LABORATORY NUMBER	DR*	ALPHA	BETA	GAMMA	γ-α	EXTINCTION	NOTES
		per cent						
Cecil†	18830	50		1.606		0 to .003		Deep chocolate-brown
	18831	50	1.602	1.607	1.607	0 to .005		Reddish brown
	18832	75		1.610		0 to .003	0°	Reddish; some grains platy
	18833	50?		1.610±		0 to .002		Red-brown; some with $\gamma - \alpha$ .010
	18834a	75?		1.577		0 to .003		Dark brown gelatinous aggregates
	18834b	100		1.581±	1.581±			Altered biotite, + 20 per cent of sample
Susquehanna	16312	100	1.564	1.573	1.573	.009	0°+	Flakes with numerous, varied inclusions
	16313	100	1.574	1.587	1.587	.013	0° – 40°	Like 16312; color brown
	17554	100	1.540	1.560	1.560	.020	0°	Good flakes; sharp extinction
	17555	100	1.522	1.541	1.541	.019	0° – 15°	Good flakes; pale; indexes variable
	17556	100	1.477	1.499	1.499	.022	0°	Indexes vary; 2V = 0°; color white

* DR = double refraction.

† The original sample of 18835 consists of somewhat disintegrated biotite-granite-gneiss. Biotite with $\beta = \gamma = 1.648$; $\alpha = 1.595$; also altered biotite with low DR and variable, usually lower indexes.

brown in color with refractive indexes lower than those of 17554. This sample has the optical properties of montmorillonite except for its higher indexes. The white clay from the depth of 11 feet (17556) has all the properties of montmorillonite and even gave fairly good uniaxial interference figures.

The compound plates of the Susquehanna colloids are made up chiefly of grains which range in size from 1 μ in diameter down to particles invisible even under high magnification. In general, these compound plates have the optical properties of negative, uniaxial, micaceous minerals, with small 2 V and X perpendicular to the plates, or nearly so. When the flakes were viewed on edge, they usually showed parallel extinction, although all the samples, except that from the 11-foot depth (17556), contained some flakes which showed inclined extinction, usually of less than 10°. In so far as the constituent particles were platy, the visible flakes may have shown "form double refraction" (1), but the close correspondence between the birefringences found and those characteristic of particular minerals indicates that "form double refraction" plays but a small part in causing the observed effects.

Although the samples from the upper horizons of this Susquehanna profile showed birefringence lower than that of the montmorillonitic clays, the values were higher than those of the kaolinitic clays. The optical properties indicate that montmorillonitic clay is a constituent of the colloid throughout this profile, although this type of clay probably constitutes only a minor part of the colloids from the upper horizons. On the other hand, the deeper horizons are predominantly montmorillonitic.

The coarse fraction of the Susquehanna soil

In view of the preceding results and the fact that the mechanical analysis (table 4) showed considerable fine sand throughout the Susquehanna profile, examination was also made of the sand fraction of this profile. The greater part of the clay and silt of the original soil was first removed by rubbing moistened samples with a rubber pestle, followed by dispersion in water without chemical treatment, and then decanting off the suspended clay and silt. This process was repeated until fairly clean sand was obtained. The results of the microscopical study were as follows:

16312 and 16313—Virtually all clear, unfrosted, colorless to rose-tinted *quartz*. Probably no feldspar present.

17554—More varied than 16312 and 16313. At least three-fourths clear, *quartz* fragments; the remainder mostly clear, colorless *muscovite* and black iron oxides. Probably no feldspar present.

17555—About four-fifths clear *quartz* like that in the more superficial horizons; also considerable fresh feldspar (chiefly *orthoclase* and *microcline*, but some *albite* and probably *andesine*), and fairly abundant *biotite*, in part fresh and in part altered to *vermiculite*.

17556—*Quartz* accompanied by flakes of fresh *muscovite* and both fresh and altered *biotite*. No feldspar seen in a small sample.

All these samples of sand consist predominantly of quartz, and that from the depth of 10 feet (17555) contains considerable feldspar and biotite also.

The presence of microcline and muscovite in the profile strongly suggests granite or gneiss as the source rock (ultimate source). It is improbable, therefore, that these sediments were derived from bentonitic materials.

X-RAY ANALYSIS

The colloidal materials have also been investigated by the X-ray diffraction method. Powder photographs were made with molybdenum Kα radiation rendered approximately monochromatic by means of zirconium oxide filters. The cassettes which were used had a radius of 8 inches and were especially designed to make possible the determination of interplanar spacings as wide as

TABLE 8

Interplanar spacings of the Cecil profile, air-dried samples

	18830 (0-6 INCHES)	18831 (8-15 INCHES)	18832 (AT 6 FEET)	18833 (AT 12 FEET)	18834 (AT 20 FEET)
K	7.25 m.	7.25 m.	7.25 m.	7.25 m.	7.25 m.
M?	4.86 v.w.	4.85 v.w.	4.9 v.w.	.....	.....
KM	4.5 s.	4.5 s.	4.45 s.	4.45 s.	4.4 s.
K	3.6 s.	3.6 s.	3.58 s.	3.57 s.	3.57 s.
Q	3.32 w.	.....	3.30 w.	.....	3.31 w.-m.
U	2.70 v.v.w.	2.70 v.w.	2.70 v.w.	2.68 v.w.	.....
KM	2.57 m. 2.52 m.	2.59 m. 2.52 m.	2.58 m. 2.50 m.	2.56 m. 2.50 m.	2.57 m. 2.51 m.
K	2.36 m.	2.38 m.s.	2.36 m. 2.32 m.	2.37 m. 2.31 m.	2.37 m. 2.32 m.
KM	2.20 v.w.	2.21 v.w.	2.18 w.	2.20 w.	.....
K	1.98 v.w.	1.98 v.w.	1.975 w.	2.01 v.w.	1.985 w.
KM	1.69 m.	1.71 m.	1.69 m.s. 1.66	1.69 m.	1.70 w. 1.655
KM	1.49 s.	1.50 s.	1.49 s.	1.485 s.	1.485 s.
K	1.455 w.	1.46 w.	1.45 w.	1.45 w.	1.45 v.w.
KM	.....	.....	1.288 w.	.....	1.286 v.w.
KM	.....	.....	1.24 w.	1.25 v.w.	1.23 v.w.

K = kaolinite, M = montmorillonite, Q = quartz, U = unidentified mineral; s = strong, m = medium, w = weak, m.s. = medium strong, v.w. = very weak.

24 Å. In the earlier work reported by Kelley, Dore, and Brown (11) the cassettes that were used did not permit observation of spacings wider than about 7 Å.

Patterns were obtained on samples of colloid from five horizons of the Cecil profile and five horizons of the Susquehanna. In tables 8 and 9 are given the interplanar spacings obtained from the film measurements. Table 10 shows a series of reference spacings for kaolinite and montmorillonite and indicates one prominent spacing of quartz. This table does not, by any means, include or agree with all of the spacings that have been reported in the literature and is not advanced as necessarily the best values for these minerals. In fact, the

selection of a reliable list of reference spacings from our own data and from those published by others has been a difficult task, partly because of the more or less heterogeneous nature of the various clay minerals which have been available for examination and partly because of uncertainties inherent in the methods that have been used. As a basis for selecting these reference lists, the authors have accepted only spacings to which Miller indexes related to a definite unit cell could be assigned. The kaolinite spacings were selected from those tabulated by Gruner (6) and indexed by him with reference to the

TABLE 9

Interplanar spacings of the Susquehanna colloids, air-dried samples

	16312 (0–8 INCHES)	16313 (8–20 INCHES)	17554 (20–30 INCHES)	17555 (AT 10 FEET)	17556 (AT 11 FEET)
M	14.1 w.	14.5 v.w.	14.5 m.	14.8 s.	15.2 s.
K	7.1 m.	7.1 m.	7.2 w.	7.2 w.	
M	4.9 v.v.w.	4.9 v.v.w.			4.9 v.v.w.
KM	4.45 v.s.	4.5 v.s.	4.45 v.s.	4.45 v.s.	4.45 v.s.
K	4.3 w.				
K	3.55 w.	3.55 w.	3.52 w.	3.52 w.	
Q	3.32 v.s.	3.32 w.	3.34 w.	3.34 w.	
M					3.02 w.
U		2.70 w.			
KM	2.58 s.	2.58 s.	2.57 v.s.	2.57 v.s.	2.54 s.
K	2.50 w.	2.50 w.	2.50 w.	2.50 w.	
K	2.34 m.s.	2.34 m.s.	2.34 w.m.	2.34 w.m.	
KM	2.23 v.v.w.	2.23 v.v.w.	2.20 w.	2.20 ?	2.23 v.w.
U	2.11 v.w.				
K	1.99 w.	1.99 v.w.	2.00 v.v.w.	2.00 v.w.	
K	1.81 m.s.				
KM	1.70 s.	1.70 s.	1.70 m.	1.70 m.	1.69 m.
K	1.53 w.m.				
M			1.50 s.	1.50 s.	1.49 v.s.
K	1.28 w.	1.28 w.	1.29 m.	1.29 m.	1.28 m.w.
KM	1.24 w.	1.24 w.	1.24 m.	1.24 m.	1.24 m.
U	1.20 v.v.w.				1.11 v.v.w.
U	1.18 v.v.w.				0.96 v.v.w.

For explanation of symbols, see footnote table 8.

monoclinic unit cell which he proposed. The cell dimensions given were: a = 5.14 Å., b = 8.90 Å., c = 14.51 Å.; $\beta = 100°12'$. The spacings listed are those calculated by Gruner, and the intensity values are the visual estimates made by him. The montmorillonite spacings were selected from the data of Maegdefrau and Hofmann (13). In a recent publication these authors refer the structure of montmorillonite to an orthorhombic cell with dimensions: a = 5.18 Å., b = 8.97 Å., with c variable according to the degree of hydration but having, for air-dried material, a value of about 14 to 15 Å. Correspondingly, all spacings of the (00l) series are also variable. Maegdefrau and

TABLE 10

Theoretical interplanar spacings of kaolinite and montmorillonite

FOR KAOLINITE [GRUNER (6)]			FOR MONTMORILLONITE [MAEGDEFRAU AND HOFMANN (13)]			FOR QUARTZ	
Spacing (*d*)	Intensity	Miller indexes	Spacing (*d*)	Intensity	Miller indexes	(Wyckoff)* spacing	Miller indexes
Å.			Å.			Å.	
			ca. 14–15	v.v.s.	(001)		
7.14	10	(002)					
			ca. 5	Variable	(003)		
			4.49	v.s.	(110) (020)		
4.397	6	(110) ($11\bar{1}$)					
4.248	3	(021)					
3.570	10	(004)					
3.33	Indistinct	($11\bar{3}$)				3.34 v.v.s.	(101)
			ca. 3.30	Variable	(005)		
2.582	5	(114) ($11\bar{5}$)	2.59	s.	(130) (200)		
2.480	5	(131) (132)					
2.38	2	(006)					
2.343	8	(132) ($13\bar{3}$)					
2.261	4	(202) ($20\bar{4}$)	2.24	v.w.	(220) (040)		
2.015	4	(222) ($22\bar{4}$)					
1.997		(134)					
1.826	2	($13\bar{6}$)					
1.671	7	(240) ($24\bar{2}$)	1.70	m.	(240) (310) (150)		
1.669		(136) ($13\bar{7}$)					
1.665		(151) ($15\bar{2}$)					
1.530	3	(137) ($13\bar{8}$)					
			1.495	s.	(330) (060)		
1.483	8	(060)					
1.481		(332)					
1.465	2	($33\bar{3}$)					
1.297	4	(1,3,$\bar{1}$0)	1.30	w.	(260) (400)		
1.235	3	—	1.245	w.	(420) (350) (170)		

* Calculated from the data given in Wyckoff's book *The Structure of Crystals*, Chemical Catalog Co., New York, 2d Edition (1931), p. 239.

Hofmann have assigned indexes to 14 lines in the montmorillonite pattern, and from these the present authors have selected the spacings which are of value for interpretion of their own data on the soil colloids. A single spacing for quartz has been included in the tabulation. Although kaolinite commonly gives an extremely weak line at about 3.3 Å., the present authors believe that this line on the X-ray films of soil colloids is more likely caused by the presence of quartz in the sample.

In tables 8 and 9, the capital letters before the numerical values for the various spacings indicate the minerals which might produce the particular spacings. It will be noted that in both series of soil colloids many of the spacings may be ascribed to either kaolinite or montmorillonite; however, after due allowance has been made for quartz and for the unidentified spacings, it is seen that the patterns of the Cecil series are preponderantly kaolinitic in type, whereas those of the Susquehanna series indicate clearly the presence of both montmorillonite and kaolinite.

Although it is interesting that the entire patterns of the colloids are in good agreement with their corresponding reference patterns, the most convincing evidence is found in the wide spacings which are present. The spacing of 14 to 15 Å. is characteristic of the air-dried form of the montmorillonite-beidellite group of clay minerals, and the spacing of about 7 Å. is equally characteristic of the kaolinite group, which includes halloysite and also the rarer kaolin minerals. The 7.25 Å. kaolinite spacing was present on the X-ray films of all the samples of the Cecil series, and the montmorillonite spacing of 14 to 15 Å. is entirely absent in every case. The purely kaolinitic nature of the Cecil colloid is, therefore, indicated. On the other hand, the Susquehanna colloids show both the 7 Å. spacing of kaolinite and the 14 to 15 Å. spacing of montmorillonite; hence, on the basis of these two spacings alone, and without reference to the more complete patterns, the conclusion may be drawn that both kaolinitic and montmorillonitic minerals are present in this profile.

Still further X-ray evidence bearing on the nature of Susquehanna colloids was obtained from the diffraction patterns of samples heated to constant weight at 100°C., 300°C., and 500°C., respectively, in addition to the pattern of the air-dried samples. Since the greater part of the OH ions of the lattice of both kaolinite and halloysite pass off as water vapor between 400°C. and 500°C., the X-ray diffraction patterns of these minerals are largely destroyed by heating the sample to 500°C. On the other hand, although the corresponding OH ions of montmorillonite may also be driven off by heat, they pass off at a somewhat higher temperature, and the crystal structure of this mineral is still stable at 500°C. As Hofmann, Endell, and Wilm (8) have shown, in the diffraction patterns of the montmorillonite types of clays, the position of the line corresponding to the widest spacing (i.e., 14 to 15 Å.) is variable depending upon the water content of the sample. This is interpreted to mean that the montmorillonitic lattice undergoes inner crystalline swelling upon hydration, and conversely that the lattice shrinks when water is driven out by heating.

The swelling or shrinkage occurs in the direction of the c axis and it is the (001) identity period that varies according to the heat treatment. Thus both kaolinite and montmorillonite show characteristic behavior when heated to different temperatures, and the X-ray examination of samples at different stages of hydration affords a means of determining the specific types of clay minerals that are present in soil colloids.

The data on the partially dehydrated Susquehanna colloids are given in table 11. These results, like those reported in table 9, give evidence of the

TABLE 11

Effect of partial dehydration on interplanar spacings of Susquehanna colloids

SAMPLE NUMBER	DEPTH	TEMPERATURE	MONTMORILLONITE SPACINGS*	KAOLINITE SPACINGS
	inches	°C.		
16312	0–8	25	14.1 w.	7.2
		100	14.1 v.w.	7.2
		300	None	7.2
		500	None	None
16313	8–20	25	14.5 v.w.	7.1
		100	13.9 v.w.	7.2
		300	None	7.2
		500	None	None
17554	20–30	25	14.5 m.	7.2
		100	14.5 m.	7.1
		300	10.0 v.w.	7.2
		500	10.1 w.	None
17555	120	25	14.8 s.	7.2
		100	14.5 s.	7.3
		300	9.8 m.	7.2
		500	10.0 m.w.	None
17556	132	25	15.2 s.	None
		100	14.0 s.	None
		300	10.3 w.	None
		500	9.5 s.	None

* See footnote table 8.

presence of montmorillonite in all these samples, and all but one contain kaolinite also. The former is indicated by the variable spacing, which becomes narrower and, in general, weaker at the higher temperatures, and the latter by the presence of the 7 Å. spacing, which remains unchanged up to 300° but disappears at 500°C.

It has been noted recently by other investigators (8), as well as by us (10), that some soil colloids contain a muscovite-like clay mineral, illite (5). Evidence for this mineral is shown in the X-ray diffraction pattern by the presence

of a spacing of 10 Å. which is unaltered by heat treatments within the temperature range that we have used in the preparation of the colloid samples. None of the colloids discussed in this paper gave any indication of the presence of this muscovite-like clay.

Reference is again made to table 8, which gives the X-ray results on the air-dried samples of Cecil colloids. Hendricks and Fry (7) gave corresponding data for several Cecil colloids, and Kelley, Dore, and Brown (11) reported similar data for one Cecil sample. The spacings reported herein agree well with the previously published results. It is interesting to note that throughout the entire Cecil profile the colloids gave almost the same X-ray diffraction pattern, and that this was unmistakably of the kaolinitic type. It should be pointed out that kaolinite and halloysite are so similar structurally that they can scarcely be differentiated in materials of this kind. No evidence was found of any other type of clay mineral in this Cecil profile. Seldom have we encountered a soil colloid which gave such a clear-cut X-ray diffraction pattern. A few faint lines not belonging to kaolinite were present on most of the films, but the faintness of these lines shows that the amounts present of minerals other than kaolinite or halloysite were small indeed. The samples which were heated to 500°C. gave virtually no pattern, the kaolinitic crystal structure being completely destroyed.

Upon inspection with a hand lens, the sample of granite-gneiss (19335) showed some evidence of partial alteration. Accordingly, a sample of the material was dispersed in water, by which means a small amount of colloid was separated from it. The air-dried form of this colloid gave a weak 7 Å. line, which line completely disappeared upon heating the sample to 500°C. The X-ray films for this material revealed no lines corresponding to montmorillonite or illite. Therefore, it is concluded that kaolinitic clay is formed at an early stage in the weathering of this granite-gneiss.

The data of table 9, representing the air-dried form of the Susquehanna colloids, are interesting. The A horizon was found to be preponderantly kaolinitic, montmorillonitic material being present in minor amounts. The B_1 horizon, although preponderantly kaolinitic, seems to contain a somewhat greater proportion of montmorillonitic clay than the A horizon. On the other hand, the B_2 and C horizons were preponderantly montmorillonitic or possibly beidellitic, minor amounts of the kaolinitic types of clay being present. Finally, the light-colored layer at 11 feet in depth proved to be almost pure montmorillonite. This sample gave no evidence of kaolinite. These facts are well brought out in table 11, which reports the X-ray data on samples heated to different temperatures.

DISCUSSION

The foregoing data on the Cecil colloids agree very well with the results of other investigators (4, 7, 9, 14). They show that these colloids are definitely kaolinitic or halloysitic in character. In this connection it is especially in-

teresting to note that granitic types of rocks appear to weather to kaolinitic types of clay under comparatively heavy rainfall both in California and in Alabama (10).

The analyses of the Susquehanna colloids agree well with those previously published by Baver and Scarseth (2, 3). The marine sediments, which are considered to be the parent material of the Susquehanna soil, seem to have contained montmorillonitic clays originally, but weathering under the climate of Alabama has apparently converted the greater part of the montmorillonite or beidellite of the superficial sediments into kaolinite or halloysite. Consider-

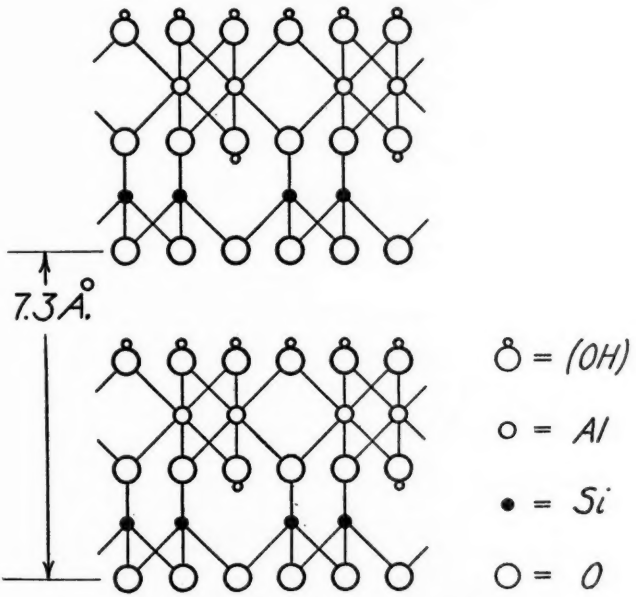


FIG. 3. THE SEQUENCE OF ATOMIC PLANES OF KAOLINITE
Distance between adjacent layers of lattices is constant irrespective of water content

ation of the composition and structure of kaolinite and of montmorillonite indicates the possibility of such a change. Kaolinite has the composition $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$, and montmorillonite $Al_2O_3 \cdot 4SiO_2 \cdot nH_2O$. The structural constitutions that have been proposed for these two minerals, as illustrated in figures 3 and 4, show that the loss from montmorillonite of one-half its silica and the accompanying hydration of the aluminum ions could lead to the formation of kaolinite. Should further research verify these results, it would follow that under intensive weathering montmorillonite is less stable than kaolinite. That the Susquehanna horizon has actually undergone rather in-

tensive leaching is indicated by the fact that the entire profile is decidedly acidic and practically free from chloride.

An alternate explanation has also been suggested, namely, that silica has been dissolved in the upper part of the Susquehanna profile and leached down

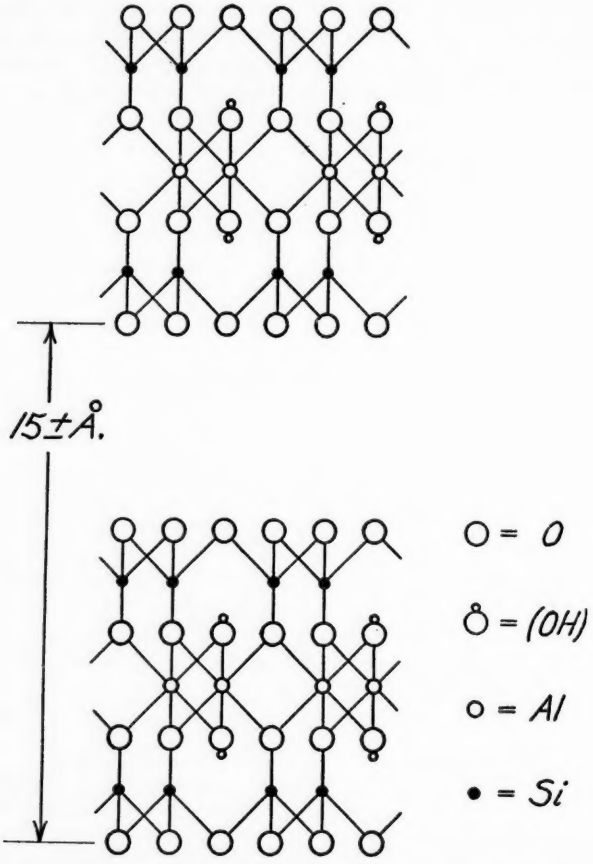


FIG. 4. THE SEQUENCE OF ATOMIC PLANES OF MONTMORILLONITE
Distance between adjacent layers of lattices is variable depending on water content

into the subhorizons, with the consequence that montmorillonitic clay has been formed in the deeper horizons as a result of the weathering of the superficial horizons. The authors of this paper are inclined to the view that the first-named hypothesis is the more probable, namely, that kaolinite or halloysite has been formed by the splitting off of silica from montmoril-

lonite, rather than that montmorillonite has been synthesized from kaolinitic types of materials.

SUMMARY

The colloidal material of the Cecil profile, discussed in this paper, has essentially the same composition throughout the profile and consists primarily of halloysite or kaolinite. Even the only slightly altered granite-gneiss found at a depth of 24 feet shows the presence of kaolinitic clay. This indicates that under the climatic conditions of Alabama, kaolinitic type of clay is formed at a comparatively early stage in the weathering of granite-gneiss.

The colloid of the Susquehanna profile is predominantly kaolinitic in the A and B horizons and beidellitic or montmorillonitic in the deeper horizons. The almost white layer found at about 11 feet in depth is primarily montmorillonite.

The above conclusions are based on chemical composition, dehydration investigations, optical properties, and X-ray analysis. All these methods of study gave consistent results.

REFERENCES

(1) Ambronn, H., and Frey, A. 1926 Das Polarisationsmikroskop. Akad. Verlagsgesellschaft, Leipzig.

(2) Baver, L. D., and Scarseth, G. D. 1931 The nature of soil acidity as affected by the SiO_2-sesquioxide ratio. *Soil Sci.* 31: 159–173.

(3) Baver, L. D., and Scarseth, G. D. 1931 Subtropical weathering in Alabama as evidenced in the Susquehanna fine sandy loam profile. *Soil Res.* 2: 288–307.

(4) Byers, H. G., Alexander, L. T., and Holmes, R. S. 1935 The composition and constitution of the colloids of certain of the great groups of soils. U. S. Dept. Agr. Tech. Bul. 484.

(5) Grim, R. E., Bray, R. H., and Bradley, W. F. 1937 The mica in argillaceous sediments. *Amer. Mineralogist* 22: 813–829.

(6) Gruner, J. W. 1932 The crystal structure of kaolinite. *Ztschr. Krist.* 83: 75–88.

(7) Hendricks, S. B., and Fry, W. H. 1930 The results of X-ray and microscopical examinations of soil colloids. *Soil Sci.* 29: 457–476.

(8) Hofmann, U., Endell, K., and Wilm, D. 1933 Kristallstruktur und Quellung von Montmorillonit. *Ztschr. Krist.* 86: 340–348.

(9) Holmes, R. S., and Edgington, G. 1930 Variations of the colloidal material extracted from soils of the Miami, Chester, and Cecil series. U. S. Dept. Agr. Tech. Bul. 229.

(10) Kelley, W. P., and Dore, W. H. 1938 The clay minerals of California soils. *Soil Sci. Soc. Amer. Proc.* 2: 115–120.

(11) Kelley, W. P., Dore, W. H., and Brown, S. M. 1931 The nature of the base-exchange material of bentonite, soils and zeolites, as revealed by chemical investigation and X-ray analysis. *Soil Sci.* 31: 25–55.

(12) Kelley, W. P., Jenny, H., and Brown, S. M. 1936 Hydration of minerals and soil colloids in relation to crystal structure. *Soil Sci.* 41: 259–274.

(13) Maegdefrau, E., and Hofmann, U. 1937 Die Kristallstruktur des Montmorillonits. *Ztschr. Krist.* 98: 299–323.

(14) Robinson, W. O., and Holmes, R. S. 1924 The chemical composition of soil colloids. U. S. Dept. Agr. Bul. 1311.

DESERT SUBSOIL TEMPERATURES

WILLIAM V. TURNAGE

Desert Laboratory, Carnegie Institution of Washington, Tucson, Arizona

Received for publication August 2, 1938

Daily changes in soil temperature do not occur at depths of 3 or more feet either in the desert or in other climates so far as is known (4). Records which show variations below this depth may very likely be erroneous (8). Weekly readings of the temperatures at these depths, therefore, are sufficient to show the salient features. Differences in subsoil temperatures in time and in space are of magnitudes small enough to demand exceptionally careful measurement of the conditions, as illustrated by the following comparison.

A mercury-in-glass thermometer was placed in an iron pipe which had been driven horizontally 3 feet into the soil through the concrete wall of a cellar at a depth of 6 feet. The flow of heat to the thermometer could take place not only through the soil but also down the concrete wall of the cellar and along the pipe. At the same depth, but some distance from the cellar, a thermocouple was placed in a small vertical hole, into which soil was then tamped. In figure 1 the two records are compared. The more rapid and more extreme heating and cooling of the thermometer exposure indicate that very appreciable errors may be introduced by improper installation. The range of the thermometer record is 35 per cent greater than that of the thermocouple, a difference great enough to make one hesitate to compare records taken in different soils or in different regions unless the conditions of measurement are known.

In the spring of 1937 several thermocouples were made, to be installed permanently at different depths in an alluvial clay at Tucson. Number 18 constantan and copper wires were used, the constantan wire and one copper lead wire being cut several feet longer than the depth at which the record was to be obtained. First one of these was covered with friction tape, then both were wrapped together for insulation and protection. These wires were then soldered together at one end, and this junction was wrapped with tape. A short piece of copper wire was soldered to the other end of the constantan wire, making a complete thermocouple. A hole $1\frac{1}{2}$ inches in diameter was bored in the soil to the desired depth, and the long, taped end of the couple was lowered into the hole. Soil was carefully tamped to fill the hole. The above-ground portion of the couple was housed in a small wooden box to protect it somewhat from the weather. Thermocouples were placed at depths of 3, 6, and 12 feet.

Readings were made weekly with a portable Leeds and Northrup galvanom-

eter. Calibrations of each couple and galvanometer as a unit were made occasionally simply by using several water baths of different known temperatures into which the above-ground junction was successively immersed, and galvanometer deflections were noted for each temperature. During the short time of the calibration the underground junction temperature, of course, remained constant. Since the relation between galvanometer deflection and temperature difference of the two junctions is linear, a simple coefficient

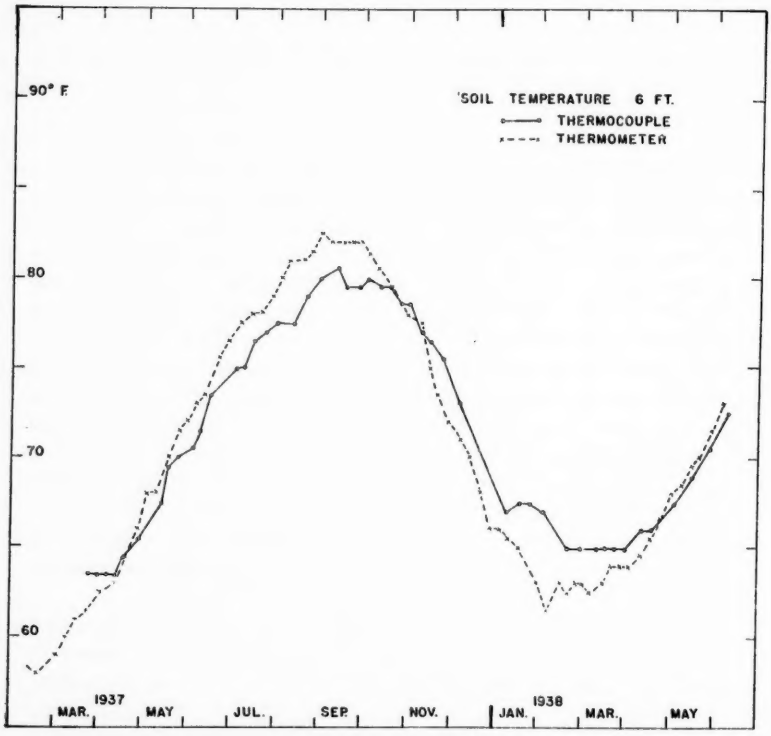


FIG. 1. THERMOMETER AND THERMOCOUPLE RECORDS IN SOIL AT A DEPTH OF 6 FEET

could be determined for each couple. This coefficient, for each couple, had also been determined before installation, and as long as the coefficient did not change, within the limits of 0.5°F. sensitivity, the couples were known to be in good condition. After 18 months the coefficient of the 12-foot couple became erratic, and very soon no definite reading of the galvanometer was possible with this couple. It was abandoned, therefore, and a new one was installed a few feet away.

In the employment of permanently placed thermocouples for subsoil tem-

perature investigations, the details to be considered include: the insulation of the junction and wires which are below ground; the occasional calibration of each couple with reference to the galvanometer, using during the test the below-ground junction as the control and varying the temperature of the above-ground junction by known amounts; the abandonment of the couple when its coefficient shows variations greater than the limits of sensitivity imposed by the instruments. The limits of sensitivity of the couples are

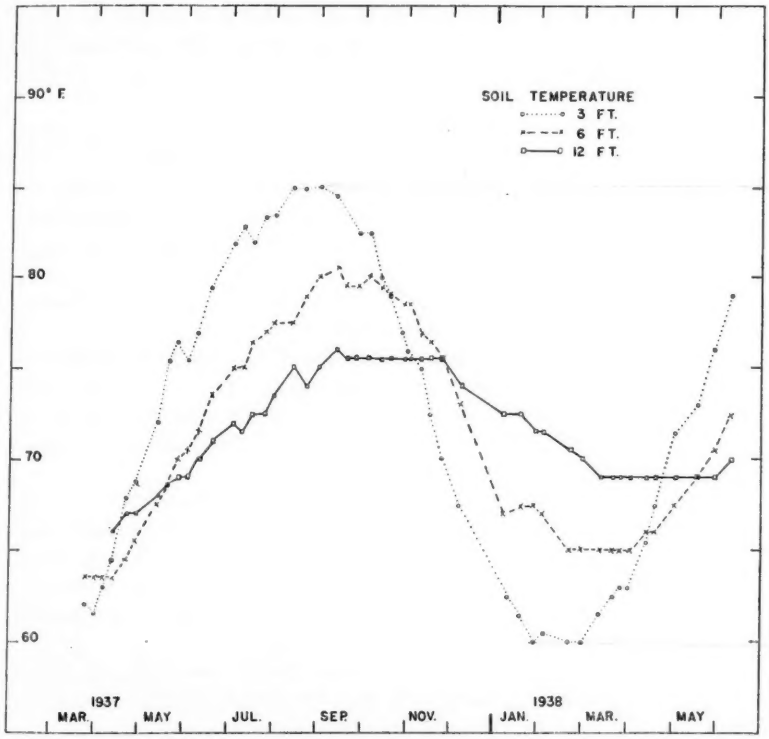


FIG. 2. SOIL TEMPERATURES, AS DETERMINED BY THERMOCOUPLES, AT DEPTHS OF 3, 6, AND 12 FEET

determined largely by the sensitivity of the thermometer used in reading the temperature of the water bath. Ease of calibration, long life of carefully made couples, minimum disturbance of natural conditions, simplicity, and small expense recommend the use of thermocouples and galvanometer for subsoil temperature investigations.

Figure 2 shows the data obtained by the thermocouples at 3, 6, and 12 feet in the bare, level, clay soil at Tucson from the spring of 1937 until the summer

of 1938. During this period the moisture content of the soil at these depths remained at approximately the wilting percentage. The first few weeks shown should probably be ignored, as some time must have elapsed after installation before true temperatures for the depths were attained.

These curves include a summer of fairly normal atmospheric conditions and a relatively mild winter. The low readings at all depths in the spring of 1937 may have been due as much to the extreme cold (of the atmospheric conditions) of the preceding winter as to the disturbance of the installation.

The maximum temperature at 3 feet, 85°F., occurred in August; at 6 feet, 80°, in September; and at 12 feet, 76°, in September. The minimum at 3 feet, 60°, occurred in January; at 6 feet, 65°, in February; and at 12 feet, 69°, in March.

The minimum temperature periods at the three depths occurred just before and during the spring growing season when leaf and stem growth of plants was rather active. The maximum temperature periods came during the summer rainy season when stem growth was at a maximum. At these depths the temperature at no time fell below the minimum for root growth of desert plants (1, 2, 3). Neither did the temperature rise as high as the optimum for root growth of the desert plants studied by Cannon, especially *Prosopis velutina*, which is the chief plant of this soil.

The range of temperature from the summer of 1937 until the summer of 1938 at 3 feet was 25°F., at 6 feet 15°, and at 12 feet only 7°. If we extend the curve showing annual range with depth, where the ratio of amplitude of successive equal intervals of depth is a constant (5), then the annual range will be reduced below 1° at 25 feet.

Since few records of subsoil temperatures exist and these have been obtained under widely dissimilar conditions, it is fruitless to push comparisons very far. The desert subsoil temperatures, when compared with those in other climates, notably those reported by Fitton and Brooks (4) and by Smith (6), do appear to have a smaller annual range, a somewhat higher maximum, a higher annual mean, and a markedly higher minimum.

The mean annual temperature at all three depths was 72.5°, somewhat higher than the 66° mean annual air temperature for Tucson reported by Smith (7).

A long thermocouple was lowered into a narrow, unused well on the grounds of the Desert Laboratory in January, 1938. The water level was 60 feet below the surface and the well was 125 feet deep. The temperature of the water was 74° throughout. Again in July, 1938, the temperature of the water in this well was 74°.

Through the courtesy of the Southern Pacific Railroad the record from a deep well at Esmond, 22 miles from Tucson, is available. Water is pumped simultaneously from two wells 20 feet apart. The water level in both is 432 feet below the surface, and one well is 883 feet deep, the other 1480 feet. In March, 1938, when the pump was started the temperature of the water

emerging from the well was 76°, 2 hours later 74°, and at the end of 4 hours 72°.

The mild and equable temperatures of the subsoil of the desert are in sharp contrast to the widely varying air temperatures, which normally range from 30° to 50° each day and from 80° to 100° annually, with a maximum of 115°. They also contrast sharply with the temperature of the surface soil, which changes from a maximum of 165° to a minimum of 20° in a year. Roots in the subsoil live in a vastly different temperature environment from that of the superficial roots and shoots of the same plant.

REFERENCES

(1) CANNON, W. A. 1911 Root habits of desert plants. Carnegie Inst. Wash. Pub. 131.

(2) CANNON, W. A. 1918 Evaluation of the soil temperature factor in root growth. *Plant World* 21: 64–67.

(3) CANNON, W. A. 1925 Physiological features of roots. Carnegie Inst. Wash. Pub. 368.

(4) FITTON, E. M., AND BROOKS, C. F. 1931 Soil temperatures in the United States. *U. S. Mo. Weather Rev.* 59: 6–16.

(5) KEEN, B. A. 1931 Physical Properties of the Soil. Longmans, Green, and Co., London.

(6) SMITH, A. 1932 Seasonal subsoil temperature variations. *Jour. Agr. Res.* 44: 421–428.

(7) SMITH, H. V. 1930 Climate of Arizona. Ariz. Agr. Exp. Sta. Bul. 130.

(8) TURNAGE, W. V. 1937 Note on accuracy of soil thermographs. *Soil Sci.* 43: 475–476.

THE INFLUENCE OF CROPPING ON THE NITROGEN-FIXING POWERS OF SOIL[1]

J. E. GREAVES AND A. F. BRACKEN[2]

Utah Agricultural Experiment Station

Received for publication August 8, 1938

The soils of Utah are generally low in nitrogen but usually carry a rich nitrogen-fixing microflora. With few exceptions, the calcium carbonate and phosphorus contents are high. When incubated, either with or without added carbonaceous material, the soils of this state usually register gains in total nitrogen. The extent of gain varies with the specific soil, the crop grown, the cultural treatments, the manure treatment, and the irrigation water applied. The purpose of the work herein reported was to measure the nitrogen fixation of untreated and variously treated dry-farm soil together with changes in the nitrogen and organic content.

Soil samples were collected from dry-land farms of Cache and Juab Valleys. Representative cropped and adjacent virgin soils were selected. Five locations approximately equidistant from one another were established on each farm, and an equal number on adjacent virgin land. For each location, five soil borings were made to a depth of 3 feet in Juab Valley and of 2 feet in Cache Valley in 1936. The five first-foot sections from each location on the cropped land were mixed together, as were those on the virgin land; the second- and third-foot sections were similarly treated. The nitrogen-fixing powers of these soils were determined by the use of the following medium:

Monopotassium phosphate	0.02 per cent
Magnesium sulfate	0.02 per cent
Sodium chloride	0.02 per cent
Calcium sulfate	0.01 per cent
Iron as ferrous sulfate	50 p.p.m.
Iodine as sodium iodide	40 p.p.m.
Manganese as manganous carbonate	40 p.p.m.
Mannitol	1.5 per cent
Distilled water	1,000 cc.

The monopotassium phosphate was dissolved separately and made slightly alkaline to phenolphthalein by the addition of 0.1 *N* sodium hydroxide.

The medium was distributed in 100-cc. portions into 500-cc. Erlenmeyer

[1] Contribution from the departments of chemistry and bacteriology and of agronomy, Utah Agricultural Experiment Station.

[2] Chemist and bacteriologist, and associate agronomist, respectively. Authorized for publication.

flasks. Each flask received 0.5 gm. of calcium carbonate, after which it was autoclaved at 130°C. for 15 minutes. Each flask was inoculated with a 1-gm. portion of the soils to be tested and then incubated at 28° to 30°C., alongside a sterile check, for 21 days. The total nitrogen was determined by the Gunning-Hibbard method. The statistical analysis was made by calculating the variance according to Fisher.[3]

In Juab Valley, seven fields were sampled, 35 composite samples from the cropped soil and an equal member from adjacent virgin land. Separate analysis was made of each sample. The averages are given in table 1. It will be observed that the difference for each foot section is in favor of the cropped land which, statistically, is highly significant. The difference in milligrams

TABLE 1

Nitrogen fixed by soil from virgin and cropped wheat land, Juab County, Utah

mgm. N per gm. soil

TREATMENT	1ST FOOT	2ND FOOT	3RD FOOT
Cropped	2.46	1.33	0.76
Virgin	1.45	0.85	0.53
Difference	+1.01	+0.48	+0.23

TABLE 2

Nitrogen fixed by soil from wheat and virgin land, Cache County, Utah

mgm. N per gm. soil

TREATMENT	1ST FOOT	2ND FOOT
Cropped	6.77	5.41
Virgin	6.23	6.22
Difference	+0.54	−0.81

of nitrogen fixed between samples taken from the various depths of 1, 2, and 3 feet was also found to be highly significant.

In Cache Valley, in northern Utah, four representative fields were similarly sampled. The figures given in table 2 are, therefore, averages of 20 composite samples. The data for the first-foot section show a difference in favor of the cropped land, but in the second foot the virgin land had higher nitrogen fixation. Although these figures reverse each other, both, statistically, were found to be highly significant.

During the fall of 1937, these same four fields together with two others in Cache County were sampled. The procedure was the same as in the previous year except that samples were taken in horizons and are designated as horizon A and horizon B. The averages from the 30 analyses are given in table 3.

[3] Snedicor, G. W. 1934 Calculation and Interpretation of Analyses of Variance and Covariance. Collegiate Press Inc., Ames, Iowa.

It is evident from the data that on these particular soils there was a greater fixation of nitrogen in the cropped than in the virgin soils. It is interesting to compare these results with those previously obtained on four of the farms. Whereas, in 1936, the cropped soil fixed the greater amount in the first foot and this was reversed in the second foot, greater fixation was obtained in the cropped soil in both horizons in 1937. In sampling during 1937 it was sometimes necessary to go to 18 to 24 inches before horizon B was reached; hence, in many cases horizon A represents soil from the first and second foot, whereas horizon B may represent soil taken from the third foot. Nevertheless, the cropped soil fixed significantly more nitrogen than did the virgin soil.

A comparison of the nitrogen-fixing abilities of alfalfa soil and virgin soil in Juab and Cache Counties is shown in table 4. The nitrogen-fixing power of the

TABLE 3

Nitrogen fixed by soil from wheat and virgin land in Cache County, Utah

mgm. N per gm. soil

TREATMENT	HORIZON A	HORIZON B
Cropped	7.81	5.84
Virgin	6.39	4.30
Difference	+1.42	+1.54

TABLE 4

Nitrogen fixed by virgin and alfalfa soil, Juab and Cache Counties, Utah

mgm. N per gm. soil

TREATMENT	1ST FOOT	2ND FOOT	3RD FOOT
Alfalfa	7.98	5.34	5.34
Virgin	3.80	2.76	0.89
Difference	+4.18	+2.58	+4.45

alfalfa soil is much greater than is that of the virgin soil. This difference in nitrogen-fixing power persists in the second-foot and even the third-foot section of alfalfa soil. There is no good evidence that the increased fixation observed when alfalfa soil is inoculated into a synthetic medium is due to Rhizobium. Consequently the conclusion is drawn that alfalfa, when grown on soil, either increases the number or the physiological efficiency of the nitrogen-fixing microflora of the soil. It is not clear whether this increased activity is due to the furnishing of a good source of carbonaceous material, the draining from the soil of its soluble nitrogen, direct action of the alfalfa plants upon the nonsymbiotic nitrogen fixers of the soil, or the excreting of organic substances which provide a source of energy for the bacteria.

In order to determine whether the increased nitrogen fixation occurring in alfalfa soils is due to the added plant residues, soil from one of the permanently

fallow plats at the Nephi Dry-Land Substation (Juab County) was kept under controlled conditions in the greenhouse. The soil was thoroughly mixed and placed in 2-gallon jars. Varying quantities and kinds of plant residues were finely ground and thoroughly mixed with the soil, which was kept bare and occasionally watered. All treatments were replicated, and duplicate nitrogen-fixation determinations were made on each pot at intervals by seeding 1 gm. of the variously treated soil into synthetic medium. Gains of nitrogen were determined after 21 days of incubation.

Alfalfa, pea vines, and straw were each added to the soil at the rates of 1, 2, 3, 4, 5, and 10 tons per acre. The soils were placed in the greenhouse June 5, 1923. The soil from each individual pot was analyzed separately for total nitrogen in 1925, 1926, and 1931. The average results are reported in table 5.

Alfalfa materially increased nitrogen fixation during the first 3 years, the extent varying with the quantity of alfalfa added. During the first 2 years, however, straw is equally effective. This makes it doubtful whether the observed stimulation noted in field soil is due to the plant residues, for if it were,

TABLE 5

Nitrogen fixed by soil treated with varying kinds and quantities of plant residues

mgm. N per gm. soil

TREATMENT JUNE 5,1923	AUG. 1925	AUG. 1926	AUG. 1931
Untreated soil	6.6	5.3	4.7
Soil + alfalfa	8.8	8.2	4.8
Soil + pea vines	6.6	5.6	5.6
Soil + straw	8.8	4.9	4.1

wheat should be equally effective in increasing nitrogen fixation. The plant residues were added only once, yet the influence of the alfalfa was perceptible 3 years later but had entirely disappeared by the end of 8 years. How long it would continue cannot be stated from this work.

Excessive quantities of straw decreased nitrogen fixation. The greatest fixation occurred when from 1 to 3 tons were added to each acre. Probably better results would be obtained if small quantities were added yearly.

Consequently, it may be tentatively concluded either that alfalfa stimulates nonsymbiotic nitrogen fixation in soil by largely utilizing the soluble nitrogen of the soil, as a result of which the nitrogen fixers are forced to use and become efficient in gathering atmospheric nitrogen, or else the plants furnish a source of energy to the soil nitrogen fixers.

In 1925, 1926, and 1934, total nitrogen determinations were made on the soil. The average results are recorded in table 6.

The soil, when placed in the pots in June 1923, contained 0.099 per cent of total nitrogen. The quantity of nitrogen carried in August 1925 was considerably greater in the legume soil than in soil to which straw had been added.

There was also an appreciable increase during the following year, 1926. This was greatest in the soil receiving alfalfa and least in the soil receiving straw. In every case, however, there was unmistakable evidence of nonsymbiotic nitrogen fixation. It has been repeatedly observed that for some time after

TABLE 6

Percentages of nitrogen in soil receiving various plant residues

TREATMENT JUNE 1923	AUG. 1925	AUG. 1926	AUG. 1934
Soil untreated	.100	.103	.102
Soil + 1 ton per acre alfalfa	.102	.118	.103
Soil + 2 tons per acre alfalfa	.115	.123	.106
Soil + 3 " " " "	.116	.121	.106
Soil + 4 " " " "	.118	.124	.110
Soil + 5 " " " "	.117	.119	.110
Soil + 10 " " " "	.121	.126	.115
Soil + 1 ton pea vines	.103	.109	.100
Soil + 2 tons " "	.110	.112	.100
Soil + 3 " " "	.111	.113	.102
Soil + 4 " " "	.114	.115	.104
Soil + 5 " " "	.112	.115	.105
Soil + 10 " " "	.118	.129	.110
Soil + 1 ton per acre straw	.102	.108	.104
Soil + 2 tons " " "	.103	.103	.105
Soil + 3 " " " "	.103	.104	.102
Soil + 4 " " " "	.102	.104	.102
Soil + 5 " " " "	.105	.108	.105
Soil + 10 " " " "	.105	.109	.103

TABLE 7

Percentages of organic carbon in soil receiving various treatments

TREATMENT	1934	TREATMENT	1934
Soil untreated	.839	Soil + 4 tons pea vines	.878
Soil + 1 ton alfalfa	.838	Soil + 5 " " "	.879
Soil + 2 tons alfalfa	.842	Soil + 10 " " "	.879
Soil + 3 " "	.860	Soil + 1 ton straw	.843
Soil + 4 " "	.863	Soil + 2 tons "	.841
Soil + 5 " "	.891	Soil + 3 " "	.842
Soil + 10 " "	.886	Soil + 4 " "	.858
Soil + 1 ton pea vines	.830	Soil + 5 " "	.882
Soil + 2 tons " "	.831	Soil + 10 " "	.885
Soil + 3 " " "	.867		

soil is well mixed, and especially when a suitable source of carbonaceous material is added, there is a measurable gain in total nitrogen, which is followed in many instances by a loss. These data show this relationship. Could this have been prevented by the occasional addition of small quantities of plant

residues and the occasional stirring of the soil? Some evidence points to this conclusion.

In 1934 the total organic carbon in the soil was determined. The average results are reported in table 7. In 1934, eleven years after the material had been added to the soil, all of the treated soils except those receiving 1 ton alfalfa, and 2 tons peas carried more organic carbon than did the untreated soil. The quantity in the soil was dependent upon the quantity added and apparently was independent of the kind. It is highly probable that most of the organic carbon which may serve as energy for the nonsymbiotic nitrogen fixers had disappeared, leaving only highly resistant carbonaceous material.

SUMMARY

When soils from wheat land and adjacent virgin land from Juab County were inoculated into a synthetic medium, the gains in nitrogen from the cropped soils were greater than those from the virgin soils in all three foot sections used; in Cache County, fixation was greater in the cropped first foot but is reversed in the second foot. The nitrogen gains made by the Cache County soils were considerably greater than those of Juab soils. This is probably due to two factors; namely, the Cache soils contain a larger quantity of carbonaceous materials than the Juab soils; almost all of the Cache soils carry a rich Azotobacter flora, whereas these organisms are generally absent in the Juab soils. When alfalfa soil was inoculated into the same medium, the fixation was about twice that observed when virgin or wheat-land soil was used. The increase is manifest in the first, second, and third foot sections of soil and is the result of either a direct or an indirect action of the legume upon the nonsymbiotic nitrogen fixers.

The addition of ground alfalfa, ground pea vines, and ground straw to the Nephi dry-farm soil increased to approximately the same extent the nitrogen-fixing power during the first 2 years. The influence of the treatment with alfalfa persisted longer, however, than did the influence of the pea vines or straw. All the plant residues caused greater increases in the nitrogen in the soil than could be accounted for by the added nitrogen; hence, the increases must have resulted from greater nitrogen fixation owing to the furnishing of energy to the nonsymbiotic nitrogen fixers. The greatest gains in soil nitrogen were caused by the addition of alfalfa. Some of the added carbonaceous material still remained in the soil after 11 years.

A BACTERIUM ANTAGONISTIC TO RHIZOCTONIA SOLANI[1]

T. C. CORDON AND C. M. HAENSELER

New Jersey Agricultural Experiment Station

Received for publication August 12, 1938

For many years investigators in the field of microbiology have recognized the significance of associative effects among microörganisms and have paid much attention to beneficial and to antagonistic reactions between certain species or groups. Waksman (7) has given a comprehensive review of the literature on this subject, and current interest in the problem is shown by the studies of Weindling and associates (10, 11, 12), of Allen and Haenseler (1), and of Daines (3) on the antagonistic action of Trichoderma toward certain fungi, and by the studies of Waksman and associates (8, 9) on the interaction between Trichoderma, Actinomyces, and certain bacteria. Thom and associates have also studied the antagonistic relationship between various organisms and the cotton root rot fungus, *Phymatotrichum omnivorum.*

Numerous cases are recorded in the literature of bacteria capable of repressing the development of certain fungus pathogens. Bamberg (2) isolated from corn plants which had been inoculated but not infected with *Ustilago zeae*, a bacterium that prevents normal infection and also destroys colonies of the smut fungus growing on artificial media. The presence of the organism was necessary: extracts had no effect. It was suggested that widespread distribution of such bacteria might bring about a check on the multiplication of the fungus in the soil. Four types of bacteria antibiotic to the smuts and certain other fungi were described by Johnson (4); some of the bacteria were found to produce enzymes which were able to dissolve the chemical constituents of the cell walls of fungus sporidia; it was shown that the bacteria act also upon specific fungi in the soil. Novogrudsky et al. (6) were able to increase the germination capacity of flax seeds infected with *Fusarium*, *Colletotrichum*, and *Fungus sterilis*, from 50.5 to 94.0 per cent by treatment with water suspensions of certain bacteria. Some of the bacteria were able to lyse the fungi, whereas others merely retarded their development. Sterile filtrates from the bacteria were equally as effective as the living cells in controlling the parasitic activity of the fungi. Bacterization was more effective in sand or solution culture than in soil.

The present investigation is concerned with the antagonistic action of a bacterium[2] toward the fungus *Rhizoctonia solani*. The bacterium was isolated

[1] Journal Series paper, New Jersey Agricultural Experiment Station, department of plant pathology.

[2] A rough strain of *Bacillus simplex*. The authors are indebted to N. R. Smith of the U. S. Department of Agriculture for identifying the organism.

from a petri plate of nutrient agar on which tomato seeds had been plated and probably was carried as a contaminant on the seed. Our attention was attracted to the bacterial colony by the strong antagonism which it showed toward various common fungi, such as Aspergillus and Penicillium, present on the same plate. The *Rhizoctonia* culture used was isolated from diseased bean seedlings, and from its cultural characteristics, as well as its parasitic habits on potatoes, was identified as *R. solani*.

EXPERIMENTAL

During preliminary experiments it was found that if the bacterium in question and the *Rhizoctonia* were planted in close proximity to each other on the same petri plate containing potato-dextrose agar, growth of the fungus was arrested several millimeters from the bacterial colony. Agar blocks taken from this "sterile zone" between the two colonies and transferred aseptically to another petri plate containing a young *Rhizoctonia* colony also prevented the fungus from developing in the immediate vicinity of the agar block (plate 1). These observations suggested that the bacterium had produced an extracellular toxic principle, which was diffusible in the agar and which was capable of inhibiting the growth of the *Rhizoctonia*.

Laboratory experiments

Experiments were then conducted to determine whether this toxic substance would be produced by the bacterium in a liquid medium and whether it could be separated from the bacterial bodies and studied more closely. The bacterium was grown in potato-dextrose solution containing 1 per cent peptone. After 4 to 6 days the cultures were filtered through a sterile Seitz filter, and the filtrate was transferred to culture flasks and inoculated with *Rhizoctonia*. The fungus failed to make any growth whatever in this filtrate, indicating that either the nutrients had been exhausted or the filtrate had been rendered toxic to *Rhizoctonia* by the bacteria. It was evident that the lack of growth was not due to the depletion of nutrients, since the addition of an equal volume of fresh nutrient to the toxic filtrate failed to restore its ability to support fungus growth, whereas fresh nutrient, diluted with an equal volume of water, supported a luxuriant growth of *Rhizoctonia*. As further proof of the abundance of available nutrients in the filtrate, it was shown that the bacterium, when reinoculated into the filtrate, grew normally.

The preliminary tests were all conducted with sterile filtrates obtained by filtering the culture through a Seitz filter. It was soon found, however, that the toxic principle produced by the bacterium was heat-stable and able to withstand autoclaving for 20 minutes at 15 pounds steam pressure without apparent loss of its toxicity toward *Rhizoctonia*. Subsequent studies were conducted with heat-sterilized filtrates.

The relative potency of the toxic principle in the filtrate from a liquid culture of the bacterium was determined by testing to what extent the toxic

solution could be diluted and still retain its ability to suppress the growth of *Rhizoctonia*. The bacterium was grown in the usual medium for 1 week, the culture autoclaved at 15 pounds steam pressure for 15 minutes, and the resultant solution diluted with unused nutrient medium so that solutions containing 0, 5, 9, 17, 23, 30, 40, 50, and 100 per cent, respectively, of the autoclaved toxic medium were obtained. These solutions were then autoclaved and inoculated with *Rhizoctonia*. The fungus failed to grow in any of these cultures except the one which contained 100 per cent of the unused medium. Even where 5 per cent of the nutrient solution consisted of the toxic bacterial medium, no *Rhizoctonia* growth was obtained. After 1 week these same cultures were reinoculated with *Rhizoctonia* to see whether the 7 days' aging had weakened or destroyed the toxic principle. Again no growth was obtained. Other experiments have shown that toxic solutions held as long as 8 weeks are still active in suppressing the fungus.

Tests were also conducted on the effect of age of the bacterial culture on the pH of the filtrate and on the concentration of the toxic principle. Measurements of pH were made by means of a glass electrode. Duplicate flasks

TABLE 1

Toxicity toward Rhizoctonia and reaction of bacterial cultures of different ages

AGE OF CULTURE.....*days*	0	1	2	3	4	5	6	7	8	10	12
Before autoclaving.....*pH*	5.95	5.63	5.55	5.97	6.18	6.45	7.45	7.94	8.37	8.58	8.68
After autoclaving.....*pH*	5.66	5.57	5.49	5.84	4.92	6.39	8.28	8.53	8.57	8.62	8.68
Lowest proportion of toxic medium effecting complete suppression of *Rhizoctonia*.....*per cent*	....	....	5	5	1	5	5	5	5	5	5

containing potato-dextrose-peptone solution were inoculated with a young culture of the bacterium on a series of successive days; at the end of the twelfth day, cultures varying in age from 0 to 12 days were obtained. The pH of each of the cultures was determined on the twelfth day. The cultures were then autoclaved, at 15 pounds pressure for 15 minutes, and pH readings were again taken. Each of the thirteen autoclaved cultures was used to make a series of five dilution cultures, containing 50, 10, 5, 1, and 0.1 per cent of the toxic medium, respectively. These diluted cultures were inoculated with *Rhizoctonia* and incubated at 28°C. The results are shown in table 1.

It will be seen from the table, that in media containing filtrates from bacterial cultures which were 1 day old or less, the *Rhizoctonia* grew at all the dilutions used, indicating that in these young cultures the toxic principle had not yet been produced in sufficient concentration to inhibit the fungus growth. The filtrates from 2- to 12-day-old bacterial cultures, on the other hand, proved to be highly toxic to *Rhizoctonia*. Fungus growth was completely suppressed in media containing only 5 per cent of the toxic filtrate, and in one case 1 per cent of the filtrate was sufficient to inhibit growth. In

other cultures, 1 per cent, and in a few cases 0.1 per cent of the filtrate caused an observable retardation in the growth of *Rhizoctonia* for several days.

In order to determine whether the *Rhizoctonia* was killed or merely suppressed by the toxic bacterial solution, those cultures which showed no visible signs of growth after 3 weeks' incubation were diluted with an equal volume of fresh medium and incubated further. In some cases, where the original medium contained only 1 to 5 per cent toxic filtrate, the fungus resumed growth after dilution. In all cases where the culture originally contained 10 per cent or more of the toxic filtrate, however, the *Rhizoctonia* inoculum failed to grow after dilution, indicating that the fungus had been killed. Further proof of the death of the fungus was obtained by removing aseptically the inocula from the toxic cultures and reinoculating them into fresh sterile medium. No growth was observed. The results indicate that if the toxic principle produced by the bacterium is sufficiently concentrated it may prove lethal to *Rhizoctonia*.

The question may be raised whether the pH of the autoclaved cultures might have some effect on the observed toxicity to *Rhizoctonia*. It is true that the 6- to 12-day-old cultures after autoclaving gave pH readings well above 8.0, which may be too high for optimum growth of the fungus. In the 3- to 4-day-old bacterial cultures, however, the pH remained approximately the same as that of the fresh medium, yet these cultures proved to be just as toxic as the older, more alkaline cultures. It seems clear, then, that the reaction of autoclaved bacterial cultures is not important in determining their toxic action.

The question may also be raised as to whether the toxic substance accumulated in the bacterial culture is a degradation product of some constituent in the complex potato-dextrose-peptone medium or an excretion product of the bacterium. This phase of the question was not studied in detail, but it was shown that a simple medium like sucrose-mineral solution with nitrate as a source of nitrogen (Czapek's) was also made highly toxic to *Rhizoctonia* by the bacteria. The bacterium grew much more slowly in this synthetic solution than in the more complex medium, but after 3 to 4 weeks of growth, the former culture solution became equally as toxic to *Rhizoctonia* as the more complex medium. The high acidity (pH 4.4) of the Czapek's solution seemed to play no part in the growth of the bacterium or in the production of the toxic principle, as was shown by the fact that adjustment of the reaction of the medium to pH 6.7 to 7.0 failed to exert any observable effect on either. These results seem to point to the fact that the elaboration of the toxic principle is intimately associated with the metabolic processes of the bacterial cell rather than with the decomposition of some of the constituents of the medium.

The chemical and physical properties of the toxic principle were not studied carefully, but a few observations were made which may aid in its recognition. Treatment of the toxic medium with activated charcoal resulted in complete inactivation of the toxic principle. When the used charcoal was added to a nutrient medium it had no injurious effect on the development of *Rhizoctonia*, indicating that the toxic principle was tightly held by the charcoal. A portion

of the toxic substance could be recovered from the charcoal by extraction with hot alcohol. Protein precipitation with alcohol or ammonium sulfate failed to remove the toxic principle from the solution. Evaporation of the toxic solution with heat at atmospheric pressure concentrated the principle in approximate proportion to the loss in volume.

Greenhouse experiments

Since such marked inhibition of growth of *Rhizoctonia* was obtained with the used bacterial medium under laboratory conditions, a number of experiments were conducted to determine whether seed decay and damping-off of seedlings caused by the same fungus could be controlled under greenhouse soil conditions by means of the bacterium or its metabolic products.

In one of these tests 56 earthenware pots, each containing 1 kgm. sandy loam soil were inoculated heavily with a young culture of *Rhizoctonia* isolated from bean seedlings. After several days, during which the fungus was allowed to

TABLE 2

Effect of soil treatment on seed decay and damping-off of cucumber and pea seedlings

SERIES NUMBER	TREATMENT	NORMAL SEEDLINGS OBTAINED AFTER 2 WEEKS, IN PER CENT OF SEEDS PLANTED	
		Cucumber	Peas
1	No treatment	35	52
2	Unused medium	65	55
3	Unused medium diluted	61	77
4	Washed bacterial cells	58	75
5	Autoclaved bacterial culture	55	80
6	Autoclaved bacterial culture diluted	87	90
7	Autoclaved bacterial culture continuous	81	90

become established, the pots were grouped into seven series of eight pots each, and each pot was treated at the rate of 200 cc. per pot as follows:

Series 1—Tap water.
2—Fresh potato-dextrose-peptone solution, full strength.
3—Fresh potato-dextrose-peptone solution, one-fourth strength.
4—Water suspension of living bacteria.
5—Potato-dextrose-peptone solution in which the bacterium had grown. Culture killed by autoclaving.
6—Same as 5, diluted to one-fourth strength with tap water.
7—Same as 5 except that the same dilution was used to keep up the moisture content during the germination period, whereas in all other series water was used for this purpose.

Cucumbers (15 seeds per pot) were planted in four pots of each series and peas (10 seeds per pot) in the other four. The results, expressed in number of seedlings remaining at the end of 2 weeks, are given in table 2.

It will be seen that the smallest number of both cucumber and pea seedlings was obtained from the untreated pots (series 1). The unused medium (series 2), and particularly the diluted unused medium (series 3), caused an appreciable increase in germination and seedling survival. The cause of this direct beneficial action by the unused nutrient medium is not clear, but it may be similar to that observed by Millard and Taylor (5), who ascribed the control of potato scab by green manure treatment to the luxuriant development of a saprophytic flora which overran the parasite. The saprophytes in this case may have been favored more than the parasites by the addition of readily available nutrients. The autoclaved culture used at full strength (series 5 and 7) was slightly toxic to the seedlings, as was shown by an observable stunting, but in series 7 and in the peas of series 5, these treatments further decreased the amount of seed decay and damping-off. The living bacterial cell suspension (series 4) and the diluted used medium (series 6) caused no direct injury to the seedlings and had a markedly favorable effect on germination and seedling survival.

Two repetitions of the above test gave results which were more erratic but which showed the same general trend.

DISCUSSION

The results obtained in the soil tests were not so outstanding as those obtained in the laboratory, but there was some indication that the bacteria used were able to produce a material that is toxic to *Rhizoctonia* under soil conditions. It was clear, however, that the action of the toxic principle in the soil test was far less pronounced than that in the flask culture tests conducted under laboratory conditions.

It would appear that under the complex conditions found in the soil some factor may have influenced the activity of the toxic substances produced by the bacteria. It may have been inactivated by soil microörganisms; or it may have been adsorbed by the soil colloids, as it was by activated charcoal; it may have been inactivated by certain chemical constituents of the soil or possibly by oxidation. Further study would be necessary to determine why the toxic principle is less effective in the soil than in test tube cultures.

The results presented are essentially in accord with those of other investigators and show, first, that certain microörganisms may produce substances which are highly toxic to certain other microörganisms, and secondly, that these substances are much more active in test tube cultures than under natural soil conditions. They suggest that direct control of a soil-borne parasite by the introduction of another specific organism or its metabolic products may have some possibilities, but that such a control program may be highly complicated because of the many factors which influence the development of the specific organism or the production, accumulation, and stability of the toxic principle. It would seem that an intensive study of the soil conditions which favor the development of certain saprophytic organisms antagonistic to plant parasites may offer more promising results than attempts to control diseases by the

direct introduction of specific organisms or their metabolic products into the soil.

SUMMARY

A bacterium identified as a rough strain of *Bacillus simplex* was found to produce a diffusible, heat-stable substance in both solid and liquid media which may inhibit growth or cause death of *Rhizoctonia solani*.

A nutrient medium in which the bacterium had grown contained the toxic principle in such concentration that it could be diluted to 5 per cent of its original volume and still completely suppress the growth of the *Rhizoctonia*.

The bacterium was able to elaborate the toxic principle from Czapek's solution as well as from the more complex potato-dextrose-peptone medium.

The toxic principle is adsorbed by activated charcoal and may be partially removed from the latter with hot alcohol.

Removal, by precipitation with alcohol or ammonium sulfate, of the major portion of the protein fraction from a nutrient medium made toxic to *Rhizoctonia* by the bacterium failed to remove or destroy the toxic principle.

Treatment of a *Rhizoctonia*-infested greenhouse soil with a potato-dextrose-peptone solution used for growth of the bacterium or with the living, washed bacterial cells without nutrient medium gave appreciable control of seed decay and damping-off in cucumbers and peas. The fresh unused potato-dextrose-peptone medium also controlled these diseases to some extent, possibly by encouraging the saprophytic microflora in the soil.

REFERENCES

(1) ALLEN, M. C., AND HAENSELER, C. M. 1935 Antagonistic action of Trichoderma on Rhizoctonia and other soil fungi. *Phytopath.* 25: 244–252.

(2) BAMBERG, R. H. 1931 Bacterium antagonistic to *Ustilago zeae*. *Phytopath.* 21: 881–890.

(3) DAINES, R. H. 1937 Antagonistic action of Trichoderma on *Actinomyces scabies* and *Rhizoctonia solani*. *Amer. Potato Jour.* 14: 85–93.

(4) JOHNSON, D. E. 1931, 1935 The antibiosis of certain bacteria to smuts and some other fungi. *Phytopath.* 21: 843–863 (1931); *Proc. Sixth Internatl. Bot. Cong.* 2: 221–222 (1935).

(5) MILLARD, W. A., AND TAYLOR, G. B. 1927 Antagonism of microorganisms as the controlling factor in the inhibition of scab by green manuring. *Ann. Appl. Biol.* 14: 202–216.

(6) NOVOGRUDSKY, O., BEREZOVA, E., NACHIMOVSKAJA, M., AND PERVIAKOVA, M. 1937 The influence of bacterization of flax-seed on the susceptibility of seedlings to infection with parasitic fungi. *Compt. Rend. Acad. Sci. U. S. S. R.* 14: 385–388.

(7) WAKSMAN, S. A. 1937 Associative and antagonistic effects of microorganisms: I. Historical review of antagonistic relationships. *Soil Sci.* 43: 51–68.

(8) WAKSMAN, S. A., AND FOSTER, J. W. 1937 Associative and antagonistic effects of microorganisms: II. Antagonistic effects of microorganisms grown on artificial substrates. *Soil Sci.* 43: 69–76.

(9) WAKSMAN, S. A., AND HUTCHINGS, I. J. 1937 Associative and antagonistic effects of microorganisms: III. Associative and antagonistic relationships in the decomposition of plant residues. *Soil Sci.* 43: 77–92.

(10) WEINDLING, R. 1932, 1934 *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopath.* 22: 837–845 (1932); 24: 1153–1179 (1934).

(11) WEINDLING, R., AND EMERSON, O. H. 1936 The isolation of a toxic substance from the culture filtrate of Trichoderma. *Phytopath.* 26: 1068–1070.

(12) WEINDLING, R., AND FAWCETT, H. S. 1936 Experiments in the control of Rhizoctonia damping-off of citrus seedlings. *Hilgardia* 10: 1–16.

PLATE 1

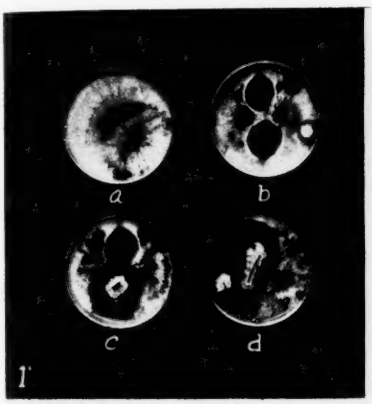
ANTAGONISTIC EFFECT OF BACILLUS SIMPLEX ON RHIZOCTONIA SOLANI

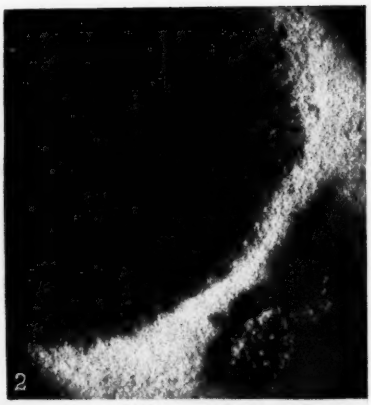
FIG. 1a. *Rhizoctonia* alone. 1b. *Rhizoctonia* with agar blocks from the zone near a bacterial colony. 1c. *Rhizoctonia* with one agar block and one bacterial colony. 1d. *Rhizoctonia* with bacterial colonies.

FIG. 2. Higher magnification of *Rhizoctonia* and agar block taken from the sterile zone near a bacterial colony.

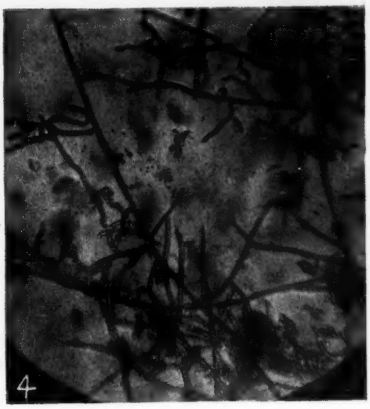
FIG. 3. Normal *Rhizoctonia* hyphae.

FIG. 4. *Rhizoctonia* hyphae in the proximity of the bacterium.


a
b
c
d
1


2

3


4

THERMOPHILIC DECOMPOSITION OF PLANT RESIDUES IN COMPOSTS BY PURE AND MIXED CULTURES OF MICROORGANISMS[1]

SELMAN A. WAKSMAN AND T. C. CORDON

New Jersey Agricultural Experiment Station

Received for publication June 30, 1938

In previous studies on the preparation of composts for the growth of the cultivated mushroom, it was found (3) that a mixture of straw and alfalfa resulted in an excellent compost, without requiring the addition of inorganic salts. Active decomposition of the materials was accompanied by a rise in temperature similar to that commonly obtained in composts of stable manure. Decomposition was particularly extensive at the higher temperatures. A number of thermophilic organisms, including bacteria, fungi, and actinomycetes, were found to be concerned in the decomposition process taking place at high temperatures. A detailed study of these fungi and actinomycetes revealed the fact that they were very similar to those commonly found in the thermophilic composts of stable manure, described in detail elsewhere (2, 4). No attempt will, therefore, be made to discuss in detail the nature and abundance of these organisms. Attention will be directed only toward their ability to break down the various organic constituents of the plant materials and to transform the latter into a dark-colored homogeneous mass, namely "humus." The effectiveness of pure cultures of thermophilic organisms, as compared with mixed populations, in the breakdown of the plant residues, and the use of the resulting composts for the growth of the cultivated mushroom will receive particular consideration.

EXPERIMENTAL

A mixture of straw and alfalfa, consisting of 60 per cent of the former and 40 per cent of the latter, on an air-dry basis, was employed in the first experiment. Thirty-gram portions of the mixed materials were placed in 500-cc. Erlenmeyer flasks and water was added to bring the mixture to 75 per cent moisture, on the total basis. The flasks were sterilized and inoculated. Pure cultures of a thermophilic Actinomyces (*Act. thermophilus*) and of a thermophilic fungus were compared with two mixed populations, one an infusion of a fertile, manured field soil, and the other an infusion of a thermophilic manure compost. Incubation took place at 50°C. for 30 days.

[1] Journal Series paper, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

The results of the first experiment (table 1) show that the thermophilic Actinomyces brought about only very little decomposition of the plant materials, as shown both by the limited reduction in the total dry matter and the comparatively small increase in nitrogen and ash. The fungus brought about more extensive decomposition, almost as much as the soil infusion, which comprised the whole soil population. The infusion of the thermophilic compost was most active in decomposing the plant residues. This points to a definite association of thermophilic organisms responsible for the decomposition of plant materials under thermophilic conditions. These results confirm the observations previously reported on thermophilic composts of horse manure (4).

A study was next made of the influence of the two plant materials used in the above compost, namely the carbohydrate-rich straw and the nitrogen-rich alfalfa, on the rate of decomposition of their specific chemical constituents under thermophilic conditions and on the quality of the compost produced.

TABLE 1

Decomposition of straw-alfalfa compost by pure cultures and complex populations of organisms in 30 days at 50°C.

INOCULUM	RESIDUAL MATERIAL, DRY BASIS	TOTAL N, PER CENT OF DRY MATERIAL	ASH
	gm.		*per cent*
Control	30.1	1.57	6.7
Thermophilic Actinomyces	28.3	1.70	7.2
Thermophilic fungus	24.4	2.31	9.0
Soil infusion	23.9	2.15	9.9
Thermophilic compost	17.2	3.51	12.9

The latter could be measured by the growth of the mushroom fungus upon the resulting compost. Three mixtures were prepared, containing straw and alfalfa in proportions of 6:4, 7:3, and 8:2. Three volumes of water were added to these mixtures of plant materials. They were inoculated with a water suspension of a thermophilic compost and incubated at 50°C. At the end of 21 and 37 days, the composts were removed and analyzed (table 2). The most extensive decomposition during the first 3 weeks took place in the 6:4 mixture; however, after 37 days, the 7:3 mixture showed the greatest decomposition. This was measured by the reduction of total dry material and by the increase in total ash and total nitrogen.

Portions of the above composts were placed in 600-cc. beakers and inoculated with spawn of a pure culture of *Psalliota campestris*. In the case of the 21-day-old composts, excellent growth of the mushroom fungus took place in the 8:2 mixture; some growth, in the 7:3 mixture; and very little growth, in the 6:4 mixture. In the case of the older composts, the 8:2 and 7:3 mixtures gave excellent growth of the spawn, but the 6:4 mixture showed no growth at all.

The chemical changes produced by the mushroom in the various composts are shown in table 6. These results emphasize the fact that the chemical nature of the compost is highly important for the successful growth of the cultivated mushroom.

In order to obtain more definite information on the decomposition of the various chemical constituents in the plant materials by thermophilic organisms, composts of straw supplemented with mineral salts were used. Thirty-gram portions of air-dry wheat straw were placed in 500-cc. flasks; 1 gm. of $(NH_4)_2SO_4$, 0.5 gm. of K_2HPO_4, and 100 cc. of tap water were added to each flask. Some flasks received 2-gm. portions of $CaCO_3$, and others did not. The flasks were sterilized under pressure and inoculated with 1-cc. portions of a suspension of spores of the thermophilic fungus grown on agar media. The flasks were incubated at three different temperatures, namely, 28°, 50°, and

TABLE 2

Decomposition of straw-alfalfa mixtures by complex thermophilic populations at 50°C.

STRAW-ALFALFA RATIO	INCUBATION	TOTAL DRY MATERIAL	TOTAL ASH	TOTAL NITROGEN
	days	*gm.*	*per cent*	*per cent*
6:4	0	90.9	5.9	1.60
6:4	21	43.8	10.6	2.61
6:4	37	40.7	11.7	3.38
7:3	0	91.0	5.3	1.30
7:3	21	48.2	9.4	2.68
7:3	37	37.6	12.7	3.17
8:2	0	91.1	4.8	1.00
8:2	21	58.3	6.9	1.53
8:2	37	45.5	9.5	1.99

65°C. As no growth occurred at the highest temperature, even after 30 days, these flasks were discarded. There was comparatively little growth at 28°C. A temperature of 50°C. proved to be optimum for the growth of the fungus. The absence of $CaCO_3$ prevented the development of the fungus almost completely, both at 28° and at 50°. A temperature of 50°C. and the addition of $CaCO_3$ proved to be most favorable for compost formation by the thermophilic fungus (table 3).

In the above experiment, the fungus decomposed, under favorable reaction and temperature conditions, 11.20 gm. of the dry plant material, or 40 per cent on an ash-free basis. The cellulose and hemicelluloses were attacked much more rapidly and in greater proportion than the total material, whereas the lignin was attacked to only a limited extent and hence accumulated; the proteins increased not only in proportion to the other constituents but even in total concentration. These results prove conclusively that the thermophilic

fungus attacked the straw in exactly the same manner as the total mixed population of a thermophilic compost.

The favorable effect of $CaCO_3$ upon the growth of cellulose-decomposing fungi was of particular interest. In order to determine whether this is true of certain mesophilic fungi as well, the results of another experiment are reported here. A comparison was made of the growth of two closely related fungi, one mesophilic and the other thermophilic, upon cellulose. A cellulose-lignin preparation was used (1) as the substrate. Five-gram portions of the air-dry material were added to 100-gm. portions of washed sand placed in

TABLE 3

Influence of temperature and $CaCO_3$ on the decomposition of wheat straw by Thermomyces

INCUBATION	$CaCO_3$ PRESENT	TEMPERATURE OF INCUBATION	TOTAL MATERIAL LEFT	PROXIMATE COMPOSITION OF RESIDUAL MATERIAL, PER CENT					
				Water-soluble organic matter	Hemicelluloses	Cellulose	Lignin	Protein	Ash
days		*°C.*	*gm.*						
.....	Control +	50	30.76	5.6	21.0	37.3	16.0	2.7	10.2
21	+	50	24.15	11.1	16.0	26.8	21.2	8.8	12.4
38	+	50	19.54	13.0	14.5	26.3	21.1	8.8	14.5
38	+	28	26.23	9.8	17.7	29.0	19.2	5.8	12.2
38	0	28	29.53	7.7	18.7	35.9	16.9	4.1	11.4
38	0	50	29.66	6.6	18.8	35.9	16.2	3.2	10.7

TABLE 4

Decomposition of cellulose in lignin-cellulose preparations by two fungi in 38 days at 28°C.

ORGANISM	$CaCO_3$ PRESENT	NITRATE-N LEFT	NITROGEN CONSUMED	CELLULOSE LEFT	CELLULOSE CONSUMED	LIGNIN LEFT
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control.....	+	75.5	.....	3,020	.....	1,369
Humicola.....	0	74.8	0.7	3,020	0	1,360
Humicola.....	+	32.9	42.6	1,209	1,811	1,436
Thermomyces.....	+	72.9	2.6	2,876	144	1,347

250-cc. Erlenmeyer flasks. One-half gram of each of the following three salts was added per flask: $NaNO_3$, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$. Moisture was adjusted to optimum. The flasks were inoculated and incubated at 28°C. for 38 days. The results (table 4) show that *Humicola*, a mesophilic organism, did not grow at all in the absence of $CaCO_3$ but attacked the cellulose extensively in its presence. The thermophilic *Thermomyces*, on the other hand, made only very limited growth at 28°C. even in the presence of $CaCO_3$. The ratio between cellulose decomposition and nitrogen consumption by *Humicola* was 42:1. The lignin was attacked by these organisms to only a very limited extent. Because of the fact that *Humicola* synthesized considerable cell

substance, the actual increase in lignin in the flasks containing this organism is due to the synthesis of some lignin-like material by the fungus.

A detailed study was next made of the decomposition of various specific organic constituents of the straw by different thermophilic organisms. A mixture of two thermophilic actinomycetes, a fungus, and the two in association were used; a mixed population from a thermophilic compost was also employed for comparative purposes. Two different sources of nitrogen were added, namely, $(NH_4)_2SO_4$ and $NaNO_3$. The preparations were sterilized at

TABLE 5

Thermophilic decomposition of wheat straw by pure and mixed cultures of microörganisms at 50°C.

Source of Nitrogen	$(NH_4)_2SO_4$					$NaNO_3$		
Inoculum	Control	Thermomyces*	Actinomycetes†	Thermomyces + Actinomycetes‡	Compost†	Control	Thermomyces*	Thermomyces + Actinomycetes‡
Chemical constituents, per cent								
Water-soluble organic matter	5.3	11.0	6.9	11.1	5.9	7.2	12.6	11.3
Hemicelluloses	19.6	14.6	16.1	14.0	12.8	22.5	15.8	14.8
Cellulose	38.4	29.4	38.1	29.0	24.3	38.5	24.4	25.8
Lignin	16.4	18.4	16.7	17.9	22.4	16.5	21.6	23.9
Ash	10.2	13.8	12.4	14.7	17.1	6.3	10.0	9.6
Protein	3.1	6.1	3.8	6.8	8.5	3.1	8.1	7.3
Total accounted for	93.0	93.3	94.0	93.5	91.0	94.1	92.5	92.7
Chemical constituents, gm.								
Water-soluble organic matter	1.67	2.74	1.92	2.63	1.22	2.10	2.45	2.13
Hemicelluloses	6.18	3.65	4.47	3.31	2.64	6.62	3.07	2.81
Cellulose	12.08	7.33	10.58	6.75	5.00	11.29	4.74	4.88
Lignin	5.16	4.58	4.66	4.24	4.62	4.83	4.19	4.52
Ash	3.21	3.44	3.45	3.48	3.52	1.85	1.94	1.81
Protein	0.98	1.82	1.06	2.21	1.75	0.91	1.57	1.38
Total found	31.5	24.9	27.8	23.7	20.6	29.4	19.4	18.9

* 42 days' incubation.
† 25 days' incubation.
‡ 17 days + 25 days.

15 pounds pressure for 2 hours. Some of the flasks were first inoculated with the fungus, and others were left uninoculated. Incubation took place at 50°C. After 17 days, some of the sterile flasks and those previously inoculated with the fungus were reinoculated with the mixture of the thermophilic actinomycetes or with an infusion of the compost. All the flasks were then incubated again at 50°C. for 25 days.

The results of this experiment (table 5) show that the thermophilic fungus was able to use the two sources of nitrogen with an equal degree of efficiency.

It decomposed the cellulose and the hemicelluloses very vigorously; it also attacked the lignin, but to a lesser extent. The actinomycetes, on the other hand, utilized the cellulose in only a limited way but attacked the hemicelluloses readily and to some extent also the lignin. The amount of protein synthesis by these organisms was rather small. The greatest amount of decomposition was brought about by the mixed population of the compost; all the constituents, including the lignin, underwent active decomposition. These

TABLE 6

Chemical changes in straw-alfalfa composts as a result of growth of Psalliota campestris

NATURE OF COMPOST		AGE OF COMPOST	PSALLIOTA INOCULUM	GROWTH OF PSALLIOTA	PROXIMATE CHEMICAL COMPOSITION, PER CENT OF DRY MATERIAL							
					Water-soluble		Hemicelluloses	Cellulose	Lignin	Crude protein	Ash	Total
Straw	Alfalfa				Organic matter	Nitrogen						
		days										
6	4	21	0*	–	6.1	0.13	14.8	26.6	21.9	14.6	10.6	94.6
7	3	21	0*	–	5.3	0.11	15.6	23.2	22.5	15.9	9.4	91.9
8	2	21	0*	–	4.1	0.08	18.1	31.9	21.8	7.9	6.9	90.7
6	4	21	+	0	7.8	0.11	11.9	23.1	23.4	12.8	11.9	90.9
7	3	21	+	++	6.5	0.12	14.5	25.0	22.5	13.6	9.4	91.5
8	2	21	+	+++	5.2	0.26	17.4	30.6	20.1	8.1	8.7	90.1
6	4	35	0*	–	4.9	0.22	12.6	17.6	25.0	14.7	11.7	86.5
7	3	35	0*	–	4.9	0.22	13.2	21.2	24.2	14.6	12.7	90.8
8	2	35	0*	–	3.8	0.13	13.9	27.2	24.1	9.8	9.5	88.3
6	4	35	0†	–	4.2	0.17	8.7	17.4	26.0	16.2	12.8	85.3
7	3	35	0†	–	7.6	0.19	10.0	17.3	24.1	15.7	11.9	86.6
8	2	35	0†	–	4.3	0.11	16.2	26.4	23.9	10.4	8.1	89.3
6	4	35	+	+	4.2	0.16	12.5	16.7	25.2	17.1	11.2	86.9
7	3	35	+	+++	10.2	0.49	12.4	16.2	22.2	14.1	11.3	86.4
8	2	35	+	++++	9.3	0.50	16.2	26.0	20.0	8.7	8.4	88.6

* Original compost, kept in a dry condition.

† Compost kept in moist condition, side by side with inoculated composts.

results prove conclusively that the thermophilic fungus is very active in the decomposition of plant materials at 50°C. The actinomycetes are less active in pure culture than is the fungus. The mixed compost population is most active, because of the supplementary activities of various organisms.

In order to illustrate the chemical changes in the organic constituents of a thermophilic compost, brought about by a secondary organism, the three composts which were obtained from mixtures of varying proportions of straw and alfalfa (table 2) were used as substrates for the growth of the cultivated

mushroom *Psalliota campestris*, as shown above. Incubation took place, at room temperature, for 4 to 5 weeks, when the contents were analyzed (table 6). Some of the beakers were then covered with a layer of soil, or cased, the surface moistened with water, and the beakers incubated further at laboratory temperature. Excellent mushroom development took place in the 8:2 composts, especially those that were 35 days old (pl. 1). Among the chemical changes produced in the compost by the mushroom, the attack upon the lignin and protein is most marked. The cellulose and hemicelluloses were hardly affected. This is brought out particularly in the two older composts, one of which was kept in a moist condition and the other inoculated with *Psalliota*.

SUMMARY

The successful preparation of composts of plant residues depends upon several factors, chief among which are the rapidity and the extent of decomposition of the plant materials. These are influenced by, (*a*) the nature of the original materials, their chemical composition, especially the proportion of nitrogenous compounds to carbohydrates; (*b*) the temperature of decomposition; (*c*) the microbiological population of the compost.

Mixtures of 80 per cent straw and 20 per cent alfalfa were found to give composts without requiring the addition of any mineral salts.

Cereal straw supplemented with mineral salts and with calcium carbonate gave an excellent compost in 21 to 35 days when kept at thermophilic temperatures, namely, about 50°C.

A thermophilic population obtained from an active compost brought about greater decomposition at higher temperatures than did a mesophilic population obtained from soil. No single pure culture could give as extensive and as rapid decomposition as the total population of a thermophilic compost.

Among the thermophilic organisms active in the decomposition of plant materials in composts, certain fungi and actinomycetes were found to play highly significant rôles.

One thermophilic fungus was found to compare favorably with the total thermophilic population.

The presence of $CaCO_3$ was essential for the rapid decomposition of plant materials as a whole and especially of cellulose by the active thermophilic fungi.

Excellent growth of the edible mushroom, *Psalliota campestris*, was obtained on composts of plant residues. This organism derived its nutrients primarily from the lignin and its transformation products and from the proteins in the compost.

REFERENCES

(1) WAKSMAN, S. A., AND CORDON, T. C. 1938 A method of studying decomposition of isolated lignin, and the influence of lignin on cellulose decomposition. *Soil Sci.* 45: 199–206.

(2) WAKSMAN, S. A., CORDON, T. C., AND HULPOI, N. 1939 Influence of temperature upon the microbiological population and decomposition processes in composts of stable manure. *Soil Sci.* 47: 83–113.

(3) WAKSMAN, S. A., AND RENEGER, C. A. 1934 Artificial manure for mushroom production. *Mycologia* 26: 38–45.

(4) WAKSMAN, S. A., UMBREIT, W. W., AND CORDON, T. C. 1939 Thermophilic actinomycetes and fungi in soils and in composts. *Soil Sci.* 47: 37–61.

PLATE 1

GROWTH OF *Psalliota campestris* ON THERMOPHILIC COMPOST OF PLANT RESIDUES

RELATIVE ABSORPTION OF NUTRIENTS BY WEEDS OF ARABLE LAND

B. N. SINGH AND L. B. SINGH

Institute of Agricultural Research, Benares Hindu University, India

Received for publication September 1, 1937[1]

It is widely recognized that weeds are very detrimental in many ways to successful farming. Among these, the crowding out of crop plants, which are overgrown and shaded or robbed of much-needed water and nutrients by the weeds, is of considerable significance. In the competition between the crops and the weeds a heavy demand is put on the limited water and nutrient supply of the growing medium, which is shared by both the crop plants and the unwanted intruders.

To understand this competition for the nutrients, we must know the nature and the amount of absorption of the various nutrients by the different weed species grown under natural conditions. This paper, therefore, deals primarily with the relative absorption of nutrients by weeds on arable land. As a preliminary to this study a series of experiments was instituted to determine the stage or stages of maximal absorption of nutrients in the life cycle of a few weeds. It was hoped that such knowledge might facilitate the further analytical program in concentrating the study on that particular stage for weeds in general—knowledge which was utilized in the present investigation.

In this paper an attempt has been made also to arrange the weeds on the basis of their relative absorption of the few essential elements. It should, however, be mentioned here that in attempting to group the weeds on the basis of certain specific characters such as their association either with the crops or with the fertilizers supplied to the field, Brenchley (3), Warrington (7), and Singh and Chalam (6) concluded that such groupings are untenable, since the methods of cultivation are the real factors that determine the dominance or the association of the different weeds.

PROCEDURE

A weedy plot nearly one third of an acre in area was thoroughly ploughed and levelled toward the end of the rainy season and was then left fallow for a complete year, thus allowing the weeds of different seasons to grow. The plot was divided into quadrats 1 meter square which were delimited by cross-wire arrangement. In each quadrat, weeds of only one species were allowed to grow, the other weeds being carefully removed by hand from time to time.

[1] Temporarily withdrawn; resubmitted September 16, 1938.

Since not all the weeds were growing at the same time of the year, a fairly large number of quadrats were left absolutely fallow by removing all the seedlings so that gradually newer species of weeds might become established in the vacant quadrats. In many instances more than one quadrat was set apart for each weed species.

Forty-nine weed species were examined. Of these, four—*Chenopodium album*, *Argemone mexicana*, *Launaea nudicaulis*, and *Oxalis corniculata*—were selected for a detailed study of their nutrient absorption under natural conditions at eight successive stages of their life span at regular 15-day intervals. The preliminary analyses demonstrated, as will be discussed later, that the maximal absorption correlated with the preflowering stage of the plant species. The bulk of the material for the majority of weeds was collected at this stage, analysis being carried out on these samples.

The weeds were dug out with roots intact and after preliminary washing and cleaning were dried in a steam oven. Aliquot samples of the intimately mixed and powdered dry material of each weed species were taken for analyses of sulfur, phosphorus, calcium, potassium, and total nitrogen. Sulfur and phosphorus were estimated gravimetrically (1), the latter being reported as phosphorus pentoxide. The perchlorate method as detailed by Mahin and Carr (4), wherein sufficient care was taken regarding the purity and the percentage of acid and alcohol used, was followed for the determination of potassium. Calcium was estimated volumetrically by titration against $KMnO_4$ solution (1) and is reported as calcium oxide. Nitrogen was determined by the modified Kjeldahl method (1).

OBSERVATIONS

Absorption of nutrient elements at different stages

Of a large number of dominant factors that determine the absorption of nutrients from the growing medium, the age of the organism plays an important part, since at some stage of its life cycle the organism is in a position to draw the maximum quantity of its food constituents.

The analytical data collected at each successive 15-day interval for the four weed species on which experiments were conducted to locate the stage or stages of maximal absorption show that the quantity of dry matter produced per plant (table 1) increases continuously throughout the entire life cycle of the weeds. It exhibits the characteristic sigmoid nature. During the earlier stages of the life cycle, the production of dry matter is highly accelerated. This continues to the flowering period but gradually slackens and reaches a virtually level phase toward the end. This is to be expected, since it is during the juvenile and the adolescent stage that the maximum development and multiplication of cells take place. Although apparently the highest quantity of dry matter is accumulated during the later stages, the rate is at its minimum during these periods.

Like dry matter production, the absorption of various nutrients is also

TABLE 1

Dry matter and absorption of nutrients per plant

AGE	DRY MATTER	CaO	S	P_2O_5	N	K_2O
days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
			Chenopodium album			
15	0.0357	0.0014	0.0002	0.0006	0.0011	0.0030
30	0.3840	0.0130	0.0021	0.0060	0.0119	0.0343
45	1.8640	0.0669	0.0096	0.0252	0.0598	0.1832
60	5.0100	0.1801	0.0267	0.0706	0.1849	0.4955
75	19.8620	0.7929	0.1023	0.2999	0.7925	1.9840
90	22.0100	0.8563	0.1134	0.3324	0.8548	2.1610
105	23.1200	0.8993	0.1172	0.3514	0.9017	2.2570
120	23.8100	0.9236	0.1195	0.3595	0.9360	2.3290
			Argemone mexicana			
15	0.0536	0.0010	0.0002	0.0007	0.0006	0.0007
30	0.1760	0.0032	0.0090	0.0234	0.0175	0.0231
45	3.9350	0.0724	0.0197	0.0531	0.0406	0.0523
60	8.6100	0.1593	0.0436	0.1154	0.0870	0.1137
75	20.0600	0.3791	0.1065	0.2728	0.2030	0.2668
90	24.1500	0.4516	0.1297	0.3371	0.2463	0.3236
105	26.0100	0.4968	0.1368	0.3381	0.2757	0.3537
120	26.0200	0.4970	0.1371	0.3383	0.2758	0.3539
			Oxalis corniculata			
15	0.0150	0.0003	0.0001	0.0002	0.0004	0.0002
30	0.0455	0.0009	0.0002	0.0007	0.0011	0.0006
45	0.1420	0.0027	0.0006	0.0021	0.0035	0.0019
60	0.2700	0.0051	0.0012	0.0039	0.0066	0.0036
75	1.0210	0.0196	0.0050	0.0153	0.0257	0.0139
90	1.3540	0.0256	0.0069	0.0198	0.0334	0.0174
105	1.3650	0.0261	0.0070	0.0201	0.0340	0.0179
120	1.3780	0.0263	0.0070	0.0201	0.0342	0.0180
			Launaea nudicaulis			
15	0.0561	0.0008	0.0003	0.0006	0.0008	0.0005
30	0.1130	0.0017	0.0006	0.0012	0.0017	0.0010
45	0.4590	0.0069	0.0023	0.0047	0.0069	0.0046
60	0.8650	0.0132	0.0046	0.0089	0.0132	0.0086
75	1.5030	0.0254	0.0080	0.0156	0.0230	0.0141
90	1.6700	0.0261	0.0089	0.0172	0.0253	0.0164
105	1.7150	0.0260	0.0092	0.0177	0.0268	0.0169
120	1.7500	0.0266	0.0093	0.0180	0.0271	0.1720

influenced by the developmental stage (table 1), as has also been observed by Ayres (2). Although a similar trend is observed in the absorption of different nutrients, the absolute quantities of the nutrients taken up by the plants at

the various stages are markedly different. It is also to be noted that both the rate and the order of absorption of the nutrients vary in different weeds.

The gain in absorption per plant of various nutrients, calculated for each successive 15-day interval, gradually increases in magnitude, except for a few fluctuations, to the seventy-fifth day. The maximum value is reached at the preflowering stage, when the plants are about to enter their reproductive period. After this, there is a gradual fall. It is seen, therefore, that the stage characterized by the formation of the flower bud, which is termed the "grand period of growth" (from the sixtieth to the seventy-fifth day), is one of high activity as far as the accumulation of salts is concerned. Such behavior must, however, be correlated with the absolute growth of the organism. Thus, on the basis of these observations, it may be concluded that it is at the preflowering stage of the weeds belonging to different genera and species that the absorption of nutrients reaches its maximum level. Further analytical work has, therefore, been much simplified by analyzing the various weed samples collected only at this stage.

Relative absorption at the preflowering stage

When the weeds are analyzed at the preflowering stage—the stage of maximum absorption—it is revealed (table 2) that most of the weeds under experimentation are exceptionally rich in their nutrient constituents and contain a much higher percentage than the common crop plants. Thus *Chenopodium album* contains 9.99 per cent potash, *Sesbania aculeata* 4.45 per cent nitrogen, and *Cassia occidentalis* 5.65 per cent calcium oxide, to cite only a few. The results are very similar to those shown by Pieters (5, p. 91) in his table XXVIII for some weed species. It is peculiar, however, that the order of concentration of the various elements as absorbed by the different weeds does not vary from weed to weed but is always more or less the same for a particular group. Some of the common weeds like *Medicago lupulina* and *Trichodesma indicum* have higher concentration of nitrogen than of other elements, whereas other weeds such as *Eclipta alba* and *Cleome viscosa* contain more calcium and potassium respectively. The data thus viewed demonstrate that the weeds may be arranged in several recognizable categories, the distinguishing feature of each of the classes being the preponderance of a particular element in the different weed species. The weeds may thus be grouped separately as those rich in nitrogen, calcium, or potash. It is difficult to separate into groups, weeds rich in phosphorus or sulfur, since the concentrations in which these two elements occur in the plant body are much below those of the other essential elements.

Weeds rich in nitrogen—The group (table 3) of weeds, the individual species of which are characterized by a high concentration of nitrogen, is the largest one, comprising 23 species belonging to 19 genera and 15 families. Some of the commonest monocotyledonous and dicotyledonous weeds are included in this group.

TABLE 2

Composition of weeds in percentage on dry weight basis

WEEDS	N	CaO	K_2O	S	P_2O_5
Argemone mexicana	1.01	1.89	1.33	0.53	1.36
Cleome viscosa	1.96	2.15	5.81	0.50	1.53
Portulaca oleracea	1.26	1.69	2.21	0.52	1.51
Portulaca quadrifida	1.16	2.12	2.33	0.50	1.56
Corchorus acutangulus	1.96	1.88	1.33	0.46	1.38
Melilotus alba	2.45	2.12	1.96	0.53	1.53
Melilotus indica	2.36	1.96	2.12	0.52	1.47
Medicago lupulina	2.13	1.61	1.45	0.54	1.39
Medicago denticulata	2.31	1.96	1.51	0.51	1.54
Sesbania aculeata	4.45	2.85	2.09	0.53	1.51
Vicia sativa	3.12	2.21	1.96	0.54	1.49
Vicia hirsuta	2.96	2.00	2.01	0.52	1.51
Lathyrus aphaca	3.56	2.19	1.94	0.53	1.51
Cassia occidentalis	3.08	5.65	2.31	0.54	1.56
Trianthema monogyna	2.01	1.91	1.12	0.49	1.39
Oldenlandia sp.	2.26	2.12	1.65	0.55	1.51
Ageratum sp.	1.96	1.29	2.41	0.53	1.56
Vernonia cinerea	2.56	2.12	3.12	0.54	1.53
Eclipta alba	1.61	1.62	1.52	0.53	1.49
Anagallis arvensis	1.55	2.12	1.99	0.51	1.56
Trichodesma indicum	2.21	2.19	1.32	0.49	1.48
Ipomoea hispida	2.15	2.06	1.86	0.50	1.53
Rungia repens	1.96	1.86	1.16	0.52	1.51
Leucas urticaefolia	2.11	2.89	2.75	0.50	1.51
Digera arvensis	3.24	4.45	3.15	0.55	1.63
Amaranthus spinosus	1.92	3.29	3.32	0.51	1.54
Amaranthus viridis	1.86	3.01	3.13	0.51	1.56
Amaranthus blitum	2.10	3.19	3.23	0.52	1.46
Achyranthus aspera	2.21	2.12	1.32	0.60	1.63
Chenopodium album	3.99	3.99	9.99	0.51	1.51
Euphorbia dracunculoides	2.12	1.92	1.63	0.52	1.55
Phyllanthus niruri	2.43	2.63	1.85	0.53	1.53
Asphodelus tenuifolius	1.99	2.41	2.31	0.61	1.65
Cyperus rotundus	1.61	1.32	1.13	0.54	1.52
Cyperus compressus	2.01	1.38	1.33	0.53	1.51
Scoparia dulcis	1.96	2.46	2.12	0.43	1.56
Sphaeranthus indica	1.54	3.86	2.85	0.51	1.12
Oxalis corniculata	2.51	1.82	1.70	0.49	1.49
Launaea nudicaulis	1.53	1.68	0.90	0.53	1.03
Convolvulus arvensis	2.02	2.11	2.00	0.51	1.01
Evolvulus alsinoides	1.36	1.26	1.12	0.53	1.09
Solanum xanthocarpum	2.56	3.36	2.12	0.56	1.63
Boerhaavia diffusa	2.01	1.93	1.12	0.50	1.54
Euphorbia hirta	1.98	1.99	1.22	0.49	1.53
Cynodon dactylon	2.08	1.58	1.22	0.50	1.01
Commelina banghalensis	2.02	2.01	1.86	0.48	1.46
Euphorbia thymifolia	2.02	2.31	1.13	0.56	1.50
Euphorbia pulcherima	1.86	2.12	1.41	0.51	1.57
Euphorbia hypericifolia	1.73	2.00	1.63	0.53	1.82

TABLE 3

*Groups of weeds, arranged in families, according to their nutrient intake**

FAMILIES	WEEDS
Weeds rich in calcium	
Papaveraceae	*Argemone mexicana*
Leguminosae	*Cassia occidentalis*
Compositae	*Eclipta alba*, *Launaea nudicaulis*, *Sphaeranthus indica*
Primulaceae	*Anagallis arvensis*
Labiatae	*Leucas urticaefolia*
Amaranthaceae	*Digera arvensis*
Euphorbiaceae	*Phyllanthus niruri*, *Euphorbia hirta*, *E. thymifolia*, *E. pulcherima*, *E. hypericifolia*
Convolvulaceae	*Convolvulus arvensis*
Solanaceae	*Solanum xanthocarpum*
Scrophulariaceae	*Scoparia dulcis*
Liliaceae	*Asphodelus tenuifolius*
Weeds rich in potassium	
Capparidaceae	*Cleome viscosa*
Portulacaceae	*Portulaca oleracea*, *P. quadrifida*
Chenopodiaceae	*Chenopodium album*
Compositae	*Ageratum* sp., *Vernonia cinerea*
Amaranthaceae	*Amaranthus spinosus*, *A. viridis*, *A. blitum*
Weeds rich in nitrogen	
Leguminosae	*Medicago lupulina*, *M. denticulata*, *Sesbania aculeata*, *Vicia sativa*, *V. hirsuta*, *Lathyrus aphaca*, *Melilotus alba*, *M. indica*
Rubiaceae	*Oldenlandia* sp.
Convolvulaceae	*Ipomoea hispida*
Boraginaceae	*Trichodesma indicum*
Nyctaginaceae	*Boerhaavia diffusa*
Amaranthaceae	*Achyranthus aspera*

* For actual values see table 2.

TABLE 3—*Concluded*

FAMILIES	WEEDS
Weeds rich in nitrogen—Concluded	
Commelinaceae	*Commelina banghalensis*
Euphorbiaceae	*Euphorbia dracunculoides*
Cyperaceae	*Cyperus rotundus* *C. compressus*
Gramineae	*Cynodon dactylon*
Geraniaceae	*Oxalis corniculata*
Acanthaceae	*Rungia repens*
Tiliaceae	*Corchorus acutangulus*
Ficoideae	*Trianthema monogyna*
Convolvulaceae	*Evolvulus alsinoides*

Of the families falling within this group, Leguminosae is the most prominent one, contributing as many as eight species belonging to five genera. Some families, such as Rubiaceae, Convolvulaceae, and Boraginaceae, contribute only one species each. Two species of Cyperaceae—*Cyperus rotundus* and *C. compressus*—are prominent monocotyledonous plants. *Cynodon dactylon* (Gramineae) also falls within this group.

Weeds rich in calcium—The group (table 3) characterized by a greater accumulation of calcium than of any other element in the plant body is the next largest one, comprising 17 weed species of 13 genera and 11 families. Of these families, Euphorbiaceae, with five species, is the most important. Of these species, four, however, are included in the single genus *Euphorbia*. The next important family of this group is Compositae with three species included under the three genera *Eclipta*, *Launaea*, and *Sphaeranthus*. Among the other families with only one species each, Leguminosae, Primulaceae, and Amaranthaceae are prominent.

Weeds rich in potassium—The group characterized by a high concentration of potassium (table 3) is the smallest one, with only nine species coming under six genera and five families. Of these families, Amaranthaceae is the most prominent, with three species included under the same genus *Amaranthus*. Compositae and Portulacaceae comprise two species each, the latter being represented by the single genus *Portulaca*. The families Chenopodiaceae and Capparidaceae have one species each.

A critical inspection of the data reveals that the majority of the representatives of a particular family fall within a single group, that is, they seem to have a characteristic avidity for some particular element as far as their intake is concerned. Thus, exclusive of *Cassia occidentalis*, which has a high calcium content, all the plants of the family Leguminosae show a greater accumulation of nitrogen than of other elements. Similarly, Euphorbiaceae exhibits a preponderance of calcium, although one species, *E. dracunculoides*, is high in nitrogen. Compositae is, however, characterized by species rich in either

calcium or potassium; the species *E. alba*, *L. nudicaulis*, and *S. indica* show avidity for calcium, and *Ageratum* sp. and *V. cinerea* are specifically rich in potassium.

DISCUSSION

The investigation shows that when the absorption of nutrients by weeds is determined by the analysis of samples collected at the preflowering stage—a stage of maximum absorption, as has been determined in this investigation by analysis at successive 15-day intervals during the life cycle of four weeds—the weeds are characteristically rich in their nutrient constituents. It is interesting to note that the concentrations in which these nutrient elements occur in the plant bodies of these weeds are much higher than those in many crop plants.

It is remarkable that in a survey of the relative nutrient absorption of the weeds certain characteristic groups become apparent, although some striking aberrant cases are noticeable. This observation is suggestive of the existence of a specific avidity of a particular group of plants for a certain ion. The aberrations generally appear when the larger systematic groups defined on morphological characteristics are taken into consideration, for weeds belonging to certain families like Amaranthaceae and Euphorbiaceae tend to fall within more than one group, whereas a review of the position of the individual genera in the different groups presents only one exception: *Euphorbia dracunculoides* comes under the nitrogen group, while all the other species of this genus are under the calcium group.

The study thus indicates the possibility of establishing a classification of plants according to their physiological behavior comparable to those systems based on macroscopic morphological distinctions. This contention finds support in the fact that all morphological and metabolic features of organisms are the definable manifestations of functions. Further data in this regard may allow the drawing of definite conclusions on the basis of which characteristic groups may be discriminated as related systematically and which may be interpreted in terms of the common phyletic origin of the members of each group.

SUMMARY

An attempt has been made to show quantitatively the relative absorption of the principal nutrient elements by weeds growing on arable land under natural conditions.

With a view to locating the stage of maximum absorption of nutrients by weeds in general, four species; namely, *C. album*, *A. mexicana*, *L. nudicaulis*, and *O. corniculata*, were analyzed and the concentrations of the different essential elements at successive stages of their life cycle were noted. The absorption per plant increases throughout the life cycle, although during the latter part the rate slackens. The gain in absorption per plant attains the

maximum in all cases at the preflowering stage of the weed, which is thus taken to be the stage of maximal absorption. The weed samples of this particular stage were utilized, therefore, for the bulk of the work.

Analytical data at the time of maximal absorption for all weed species reveal that the different elements are absorbed in different quantities, some elements being in higher concentration than others. On the basis of this observation, the weed species have been arranged into three distinct categories, the distinguishing feature of each group being the preponderance of a particular element. Of the three groups—weeds rich in nitrogen, weeds rich in calcium, and weeds rich in potassium—the first is the largest and the last is the smallest.

It is revealed that most of the members of a certain family possess a greater affinity for a particular ion than for other ions. This is especially true, though observed more or less in all the species of a family, when individual genera are considered.

The possibility of classifying plants according to their physiological behavior is indicated. Further data in this regard may reveal phyletic relationship interspersed in such physiological groupings.

REFERENCES

(1) Association of Official Agricultural Chemists 1930 Official and Tentative Methods of Analysis, ed. 3, Washington, D. C.

(2) Ayres, A. 1936 Effect of age upon the absorption of mineral nutrients by sugar cane under field conditions. *Jour. Amer. Soc. Agron.* 28: 871–886.

(3) Brenchley, W. E. 1911–1913 The weeds of arable land in relation to the soils on which they grow. I, II, III. *Ann. Bot.* 25: 155–165 (1911); 26: 95–109 (1912); 27: 141–166 (1913).

(4) Mahin, E. G., and Carr, R. H. 1923 Quantitative Agricultural Analysis. New York.

(5) Pieters, A. J. 1927 Green Manuring: Principles and Practice. Chapman and Hall, London.

(6) Singh, B. N., and Chalam, G. V. 1937 A quantitative analysis of the weed flora on arable land. *Jour. Ecol.* 25: 213–222.

(7) Warrington, K. 1924 The influence of manuring on the weed flora of arable land. *Jour. Ecol.* 12: 111–126.

THE ZINC CONTENT OF WEEDS AND VOLUNTEER GRASSES AND PLANTED LAND COVERS[1]

L. H. ROGERS,[2] O. E. GALL, AND R. M. BARNETTE[3]

Florida Agricultural Experiment Station

Received for publication July 29, 1938

"Land resting"—alternating periods of arable cultivation with one or more years' rest of the soil—has been considered good agricultural practice for many centuries. The substitution of definite sown crops of selected grasses and clover (crop rotation) for the indefinite mixture of weeds and grasses is regarded as one of the greatest improvements in agriculture (18, p. 362–363). The use of a so-called fallow of weeds and volunteer grasses or the practice of "land resting" has, however, a more or less definite place in the economical culture of some crops under certain soil conditions.

Numerous workers have studied the effects of crop rotation and resting with various crops. Garner, Lunn, and Brown (7) in addition to reviewing the literature on this subject have conducted extensive experiments with tobacco, from which they conclude, "at the present time the only system of soil management known to be effective in restoring to normal the yield of tobacco which has declined under intensive methods is the simple expedient of allowing the soil to remain idle for a period of years." Brown and Lunn (5), Brown and McMurtrey (6), Lunn (8, 9, 10, 11, 12), and Lunn and Mattison (13) have extended these studies with essentially the same conclusions.

In the general farming section of central and north central Florida, where very sandy soils predominate, the practice of "land resting" has been followed for many years. Indeed, the practice has resolved itself into a type of rotation, with weeds and grasses volunteering for one or two years followed by corn interplanted with peanuts or with velvet beans or with both. The crops are seldom fertilized, and when the corn has been broken from the stalks, the peanuts and velvet beans are usually harvested through livestock.

In instances where the sandy soils of this section have been planted to soil-depleting crops a physiological disease of corn locally called "white bud" has developed and has materially reduced plant growth and grain yields. In 1935, Barnette and Warner (4) described the physical symptoms of white bud of corn and definitely proved that it was due to a deficiency of available

[1] Published with the permission of the director of the Florida Agricultural Experiment Station.

[2] Present address, Cornell University, Ithaca, N. Y.

[3] Spectrographic analyst, assistant chemist, and formerly chemist (deceased), respectively.

zinc in the soil. Previously, Allison (1), Allison, Bryan, and Hunter (2), and Allison and Hunter[4] had obtained responses to zinc (as well as other elements) on the raw peat soils of the Florida Everglades with corn, peanuts, and many other crops. Mowry (14) and Mowry and Camp (15), also, had proved that "bronzing" of tung trees, a physiological disorder of *Aleurites fordii* and related species, grown in areas near the fields producing white bud of corn, was due to a deficiency of available zinc in the soils.

In 1936, Barnette et al. (3) published a more complete report of experiments conducted with the use of zinc sulfate under corn and other field crops grown on the sandy soils of central and north central Florida. The physical symptoms of zinc deficiency in corn, millet, velvet beans, cowpeas, and oats were described in detail. The value of zinc sulfate for preventing the development of these plant disorders and for increasing yields of these crops and others not developing definite physical deficiency symptoms was shown.

Since pure zinc sulfate was used in these experiments, the specific action of zinc in preventing the development of the physiological diseases of the several plants and in increasing plant growth was established beyond doubt. Other methods of preventing the development of white bud and of the associated deficiencies of zinc in other plants were also studied.

Thus, in the same study (3), observations were reported on a field of Norfolk and Hernando fine sands which is being used by the agronomy department of the experiment station for a "land resting" experiment. Previous to the initiation of the experiment the field had grown corn which was uniformly and severely affected with white bud. In 1933, when the experiment was started, the field was divided into four tiers of equal dimensions. Each tier was subdivided into five plots 35 feet wide and 740 feet long, and the following rotation of land covers and cover crops was practiced: plot 1—2 years of weeds and volunteer grasses followed by corn interplanted with peanuts; plot 2—1 year of weeds and volunteer grasses followed by corn interplanted with peanuts; plot 3— a mixture of *Crotalaria striata*, *C. spectabilis*, and *C. intermedia* followed by corn interplanted with peanuts; plot 4— corn interplanted with peanuts and crotalaria grown annually with a winter cover of rye or oats; and plot 5— corn interplanted with peanuts grown annually. From observations of the corn grown on these plots, Barnette et al. (3, p. 16) wrote:

> . . . the results show that "resting the land" or permitting it to lie fallow to volunteer weeds and grasses has reduced materially the percentage of white bud corn plants in the plots. The incorporation of a relatively heavy planted cover crop of crotalaria had not been as effective as fallow in reducing white bud of corn. The corn on the plots planted to corn and peanuts annually showed by far the largest percentage of white bud.

The object of the present study was the determination of the zinc content of the weeds and volunteer grasses on the "rested" plots and of the planted covers on the plots under continuous cultivation.

[4] Allison, R. V., and Hunter, J. H. Response of the peanut plant to treatment of the raw, sawgrass peat of the Everglades with a mixture of copper and zinc salts. Unpublished paper presented to Amer. Assn. Adv. Sci., New York City, December, 1928.

EXPERIMENTAL PROCEDURE AND RESULTS

In the fall of 1934, samples of plant materials were collected from plots 1 and 4 of the "land resting" experiment described in the foregoing section. Thus, samples of weeds were collected from the plots in the second year of a 2-year "rest" and of *Crotalaria spectabilis* from the plots which were planted annually to corn, peanuts, and crotalaria. The tops of the plants were broken off by hand and placed in clean paper sacks. Care was taken to avoid contamination in collecting and preparing the samples for analysis.

The plant materials were dried at 100°C., broken into smaller pieces by hand, and the percentage ash was determined by ignition in a platinum dish at a temperature not exceeding 450°C. The ash was homogenized by grinding in an agate mortar with an agate pestle. A spectrographic method (16) was used for the microdetermination of zinc in the plant ashes. The probable error of this method has been shown to be 10 per cent or less (17).

The zinc content of the plant tops expressed in parts per million of oven-dried materials of six species of weeds collected from soils "rested" for 2 years and of *Crotalaria spectabilis* from soils in annual culture to interplanted corn, peanuts, and crotalaria is given in table 1.

The most striking observation from these data is the predominately higher zinc content of all the weeds in comparison with *Crotalaria spectabilis*. The six species of weeds averaged 140 p.p.m. of zinc in the dry matter, and *Crotalaria spectabilis*, only 8 p.p.m. There is a relatively wide variation in the zinc content of the same species. The samples of Diodella had a range of 139 to 714 p.p.m. of zinc; Richardia, 22 to 73 p.p.m.; Isopappus, 31 to 48 p.p.m. These samples may be expected to vary in zinc content, however, as they were collected from widely separated parts of the experimental field.

In the fall of 1935, samples of plant materials were collected from plots 2 and 3 of the "land resting" experiment. Thus weed and grass samples were obtained from soils which had been "rested" for 1 year and from soils which had grown a mixture of *Crotalaria striata*, *C. spectabilis*, and *C. intermedia* for 1 year. In both instances the system of management had been in effect for 3 years.

Samples of the plant materials were collected, dried, ashed, and analyzed for zinc as described above. The zinc content of eight species of weeds and grasses collected from soils "rested" for 1 year and of three species of crotalaria is given in table 2.

Differences between the zinc content of the weeds and grasses collected from the 1-year "rest" plots and that of the crotalarias were not so great as those found in table 1, but the average results indicate a definitely higher zinc content in the weeds and grasses than in the sown cover crops. The average for the weeds and grasses in table 2 was 70 p.p.m. in comparison with 21 p.p.m. for the crotalarias. Even excluding the abnormally high value obtained with the foxtail sample, the weeds and grasses averaged 38 p.p.m. of zinc. A relatively wide variation in the zinc content of the samples of the same species collected from different parts of the field is noted again.

Comparison of the analyses of the same plants in tables 1 and 2 shows that, as a rule, the weeds (Diodella, Richardia, Isopappus, and fleabane) collected from the 2-year "rest" plots (table 1) had a higher zinc content than those from the 1-year "rest" plots (table 2). On the other hand, the zinc content of *Crotolaria spectabilis* sown in a 2-year rotation with corn and peanuts (table 2)

TABLE 1

Zinc content of six species of weeds collected from soils "rested" for 2 years and of C. spectabilis *from soils in annual culture to corn, peanuts, and crotalaria*

LABORATORY NUMBER	COMMON NAME OF PLANT	BOTANICAL NAME OF PLANT*	Zn† CONTENT
			p.p.m.
		Weeds	
898a	Diodella	*Diodella teres* (Walt) Small	489
899a			150
900a			139
901a			714
906a	Richardia	*Richardia scabra* St. Hil.	41
907a			28
908a			73
909a			22
910a	Isopappus	*Isopappus divaricatus* (Nutt) T & G	42
911a			31
912a			48
914a	Rabbit tobacco	*Gnaphalium obtusifolium* L.	114
918a	Fleabane	*Leptilon canadense* (L.) Britton	45
922a	Polypremum	*Polypremum procumbens* L.	31
Average			140
		Planted summer cover crops	
902a	Crotalaria	*Crotalaria spectabilis* Roth.	4
903a			8
904a			11
904b			7
905a			10
Average			8

* Identification of plants by Erdman West, mycologist.

† Concentration of zinc in oven-dried plant materials.

was definitely higher than that of this cover crop interplanted annually with corn and peanuts (table 1).

Careful examination of the weed cover of the plots showed that Diodella and ragweed were the most abundantly distributed plants on the "rested" plots, particularly those "rested" for 1 year. In general, the plants which predominate in the weed cover of the 1-year "rest" plots also are most abun-

dant on the 2-year "rest" with some minor ecological changes. As a rule, Richardia (Mexican clover or Florida pusley) is the dominant late fall land

TABLE 2

Zinc content of eight species of weeds and grasses collected from soils "rested" for 1 year and of C. striata, C. spectabilis, *and* C. intermedia *from soils planted in a 2-year rotation with corn and peanuts*

LABORATORY NUMBER	COMMON NAME OF PLANT	BOTANICAL NAME OF PLANT*	Zn† CONTENT
			p.p.m.
		Weeds and volunteer grasses	
1243	Richardia	*Richardia scabra* St. Hil.	12
1251	Foxtail	*Setaria lutescens* (Wiegel) F. T. Hubb	585
1252	Fleabane	*Leptilon canadense* (L.) Britton	30
1253			42
1256	Diodella	*Diodella teres* (Walt) Small	120
1257	Buzzard grass	*Heteropogon melanocarpus* (Ell.) Benth	100
1263	Heterotheca	*Heterotheca subaxillaris* (Lam) Britton & Rusby	13
1264			17
1265			14
1266	Isopappus	*Isopappus divaricatus* (Nutt) T & G	17
1267			17
1268			19
1269			23
1270	Ragweed	*Ambrosia elatior* L.	60
1271			65
1272			42
1273			17
Average			70
		Planted summer cover crops	
1246	Crotalaria	*Crotalaria striata* D. C.	14
1247			4
1248			20
1258	Crotalaria	*Crotalaria spectabilis* Roth.	18
1259			32
1260			21
1261	Crotalaria	*Crotalaria intermedia* Kotschy	43
1262			20
Average			21

* Identification of plants by Erdman West.

† Concentration of zinc in oven-dried plant materials.

cover of the very sandy types of the Norfolk, Hernando, and related soil series when they are planted annually to corn and peanuts or to other crops. On these same soils the annual sowing of the crotalarias is, in many instances,

unsuccessful after the first 2 or 3 years. Weeds and grasses offer competition to weakened crotalaria plants. The exact nature and cause of the failure of the crotalarias to produce annually on the sandy soils has not been determined, though many attempts have been made to ascertain the reason.

During the first several years of the "land resting" experiment, the crotalarias yielded approximately 2 tons of oven-dried material an acre, whereas weeds and volunteer grasses yielded approximately 1 ton. Despite the higher tonnage and nitrogen content, however, the planted summer cover crop of the crotalarias was not so effective in preventing the development of white bud of corn as were the volunteer weeds and grasses.

SUMMARY

Previous studies on the beneficial effects of "land resting" and on the use of zinc sulfate in preventing the development of white bud of corn suggested a study of the zinc content of indigenous and planted cover crops. Samples of 10 species of weeds and volunteer grasses and of the planted summer cover crops of three species of crotalaria were collected in the fall from the plots of a "land resting" experiment conducted on Norfolk and Hernando fine sands. By the use of a spectrographic procedure, the zinc content of the ashes of these materials was determined. The dry matter of the weeds collected from plots "rested" for 2 years averaged 140 p.p.m. of zinc; that of *Crotalaria spectabilis* planted annually, 8 p.p.m. The dry matter of weeds and grasses collected from plots "rested" for 1 year averaged 70 p.p.m. of zinc; that of three species of crotalaria planted in plots in a 2-year rotation with corn and peanuts, 21 p.p.m. These data seem to indicate that the weeds and volunteer grasses are able to absorb much larger proportions of zinc than are planted land covers and apparently make available sufficient zinc to prevent the development of white bud of corn.

REFERENCES

(1) ALLISON, R. V. 1928 [Responses of peanuts to copper and zinc.] *Fla. Agr. Exp. Sta. Ann. Rpt.* 1928: 112 R—114 R.

(2) ALLISON, R. V., BRYAN, O. C., AND HUNTER, J. H. 1927 The stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulphate and other chemicals. Fla. Agr. Exp. Sta. Bul. 190.

(3) BARNETTE, R. M., CAMP, J. P., WARNER, J. D., AND GALL, O. E. 1936 The use of zinc sulfate under corn and other field crops. Fla. Agr. Exp. Sta. Bul. 292.

(4) BARNETTE, R. M., AND WARNER, J. D. 1935 A response of "chlorotic" corn plants to the application of zinc sulfate to the soil. *Soil Sci.* 39: 145–159.

(5) BROWN, D. E., AND LUNN, W. M. 1925 Cropping tests with tobacco. Md. Agr. Exp. Sta. Bul. 275.

(6) BROWN, D. E., AND MCMURTREY, J. E., JR. 1934 Value of natural weed fallow in the cropping system for tobacco. Md. Agr. Exp. Sta. Bul. 363.

(7) GARNER, W. W., LUNN, W. M., AND BROWN, D. E. 1925 Effects of crops on the yields of succeeding crops in the rotation, with special reference to tobacco. *Jour. Agr. Res.* 30: 1095–1132.

(8) LUNN, W. M. 1932 Tobacco investigations. *S. C. Agr. Exp. Sta. Forty-Fifth Ann. Rpt.* 1932: 119–120.

(9) LUNN, W. M. 1933 Tobacco investigations. Effects of weeds. *S. C. Agr. Exp. Sta. Forty-sixth Ann. Rpt.* 1933: 141–142.

(10) LUNN, W. M. 1934 Tobacco investigations. Tobacco after weeds. *S. C. Agr. Exp. Sta. Forty-seventh Ann. Rpt.* 1934: 106–107.

(11) LUNN, W. M. 1935 Tobacco investigations. Tobacco-weed rotation. *S. C. Arg. Exp. Sta. Forty-eighth Ann. Rpt.* 1935: 107.

(12) LUNN, W. M. 1936 Tobacco investigations. Tobacco-weeds rotation. *S. C. Arg. Exp. Sta. Forty-ninth Ann. Rpt.* 1936: 95–97.

(13) LUNN, W. M., AND MATTISON, J. R. 1937 Tobacco progress report. Tobacco rotations. *S. C. Agr. Exp. Sta. Fiftieth Ann. Rpt.* 1937: 109–110.

(14) MOWRY, H. 1933 Propagation, planting and fertilizing tests with tung oil trees. *Fla. Agr. Exp. Sta. Ann. Rpt.* 1933: 91–95.

(15) MOWRY, H., AND CAMP, A. F. 1934 A preliminary report on zinc sulphate as a corrective for bronzing of tung trees. Fla. Agr. Exp. Sta. Bul. 273.

(16) ROGERS, L. H. 1935 Spectrographic microdetermination of zinc. *Indus. and Engin. Chem., Analyt. Ed.* 7: 421–422.

(17) ROGERS, L. H., AND GALL, O. E. 1937 Microdetermination of zinc. *Indus. and Engin. Chem., Analyt. Ed.* 9: 42–44.

(18) RUSSELL, E. J. Soil Conditions and Plant Growth, ed. 6. Longmans, Green and Co., New York.

BASE EXCHANGE IN SOILS: I. A CRITICAL EXAMINATION OF THE METHODS OF FINDING BASE-EXCHANGE CAPACITY OF SOILS

AMAR NATH PURI AND H. L. UPPAL

Irrigation Research Institute, Lahore, India

Received for publication October 8, 1937

Next to lime requirement, no other soil constant, perhaps, is so widely used and yet so little understood as base-exchange capacity. The term "base-exchange capacity" is frequently used in the same sense as "saturation capacity" and "adsorption capacity," and we even read of "base-exchange content" in soil literature.

The importance of base-exchange studies in soil problems is well recognized. At this stage of our knowledge, however, it is necessary that the various terms used in soil literature should be clearly defined and the principles underlying the determinations of the various soil constants understood as far as possible. The confusion about "base-exchange capacity" and other terms denoting the same thing has been chiefly due to the domination of the idea that bases are taken up by soils through adsorption; though it must be admitted that this belief is dying out.

In order to appreciate the exact significance of base-exchange capacity we may well examine a few typical titration curves of soils, as given in figure 1. These show that the base taken up by a soil is a function of its pH value. The base-exchange capacity of a soil, therefore, has no meaning unless we take it to a pH of 10 or so, that is, up to the point where the soil takes up the maximum amount of bases. Alternatively, the only logical way of expressing base-exchange capacity would be to specify the limiting pH value. We could then speak of the exchange capacity of a soil to pH so and so.

This point was recognized by Csiky (2), who recommended the replacement of adsorbed cations by a replacing agent with well-established pH, the treatment being continued until the original pH value of the reagent is reached. No method was suggested, however, for achieving this in practice. Schofield (9), perhaps for the first time, laid stress on this point and described a method of finding base-exchange capacity to pH 7. His method consists in shaking the soil with dipotassium hydrogen phosphate and measuring the decrease in the concentration of potassium ions in solution from the change in electrical conductivity of the solution. The method rests on the assumption that all the exchangeable Ca and Mg ions originally attached to the soil are precipitated as insoluble phosphates. This is obviously not correct, as the precipitation of calcium phosphate is not complete at pH 7. Besides, this method is not

applicable to calcareous soils or to alkali soils rich in exchangeable Na. Schofield also suggested the use of K_2CO_3 for the same purpose. This has the advantage of taking the soil to a higher pH value and consequently nearer to the saturation capacity. The two methods, however, would give entirely different values. Unless we know exactly what we want and what are the precise reactions taking place in a particular method, any one method may be just as good as another.

The various methods of finding base-exchange capacity may be dealt with under two heads: leaching with neutral salts and leaching with hydrolyzed salts.

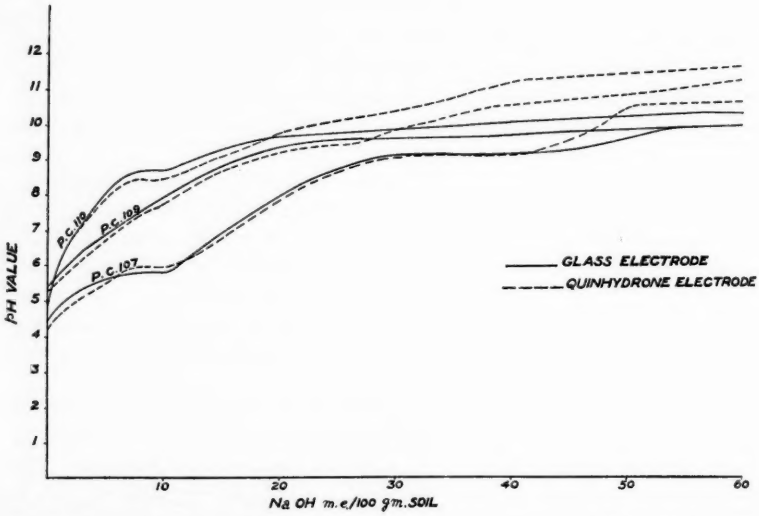


FIG. 1. TITRATION CURVES OF SOILS WITH THE QUINHYDRONE AND THE GLASS ELECTRODE

LEACHING WITH NEUTRAL SALTS

It is not generally appreciated that a neutral salt solution, not being buffered, immediately takes up the pH value of the soil, leaving it in almost exactly the same state of saturation, provided it is not too acid or alkaline. Thus, barring highly acid or alkaline soils, the base-exchange capacity thus found is approximately equivalent to the total exchangeable bases. This will be clear from table 1, which gives the so-called base-exchange capacity for the same soil containing increasing amounts of exchangeable K. The soil used in this case was a black cotton soil of high base-exchange capacity which was made unsaturated by 0.05 *N* HCl treatment. To this acid-treated soil increasing amounts of KOH solution were added and kept for several days to attain equilibrium. These soils were then leached with 1 liter of 0.2 *N* KCl solution in 100-cc. lots, and exchangeable K was determined by the ammonium car-

bonate method. It will be seen that the base-exchange capacity is dependent on the initial amount of the base present in the soil and, indeed, between pH 6 and 8 comes out to be very nearly the same as the base present in the soil. Since most of the agricultural soils are within this pH range, base-exchange capacity as determined by leaching with a neutral salt cannot be a fixed constant for the soil but must vary with the amount of the exchangeable base already present in it. The difficulty of replacing all the calcium in a soil by simple leaching with a neutral or hydrolyzed salt is not generally recognized. It was shown in a previous publication (7) that leaching with even 2 liters of a salt solution does not remove all the exchangeable Ca. This difficulty is so much greater with humus soils that it is virtually impossible to replace all the Ca with a reasonable amount of leaching. This point is brought out in a striking manner if we determine exchangeable Ca by one of the single-treatment methods previously described. The total exchangeable

TABLE 1

The base-exchange capacity of a soil with increasing amounts of exchangeable base

EXCHANGEABLE BASE	pH	EXCHANGEABLE K IN SOIL AFTER KCl TREATMENT
m.e./100 gm.		*m.e./100 gm.*
0	3.5	45.2
8	4.98	45.5
16	4.97	47.0
24	5.32	41.0
32	5.72	42.6
40	5.98	43.6
48	6.64	50.6
56	7.52	56.9
64	7.76	60.4
80	10.01	66.9
88	10.40	67.2

bases thus found are invariably more than the base-exchange capacity determined by leaching with a neutral salt (table 2).

The complication introduced by exchangeable H in acid soils has led some authors to suggest a preliminary treatment with alkali before the neutral salt treatment. Unfortunately, however, this treatment has been recommended only for acid soils and consequently leaves such soils too highly alkaline. This alkalinity gradually is leached out on subsequent treatment with a neutral salt, and the final pH value, which really determines the base-exchange capacity, is, therefore, partly dependent on the amount of leaching. Undoubtedly, this can be restricted to a definite amount, but not all soils will respond equally to this arbitrarily fixed leaching.

LEACHING WITH HYDROLYZED SALTS

Hydrolyzed salts, generally acetates, are more popular than neutral salts for leaching. Naturally they leave the soil at a higher pH value than neutral

salts and consequently give a higher value for base-exchange capacity. Ammonium acetate is extensively used for this purpose and leaves the soil generally above the neutral point. This method has one drawback in the case of humus soils, namely, a part of the humus is leached out with ammonium

TABLE 2

Base-exchange capacity and pH, as determined by various methods

Total exchangeable bases, base-exchange capacity, and titration value expressed in m.e. per 100 gm. soil

SOIL NUMBER	CLAY	TOTAL EXCHANGEABLE BASES	T/2 VALUE	INITIAL pH OF SOIL	TITRATION VALUE	CHAPMAN AND KELLEY			KELLEY			PARKER		
						pH	Base exchange capacity	Titration value	pH	Base exchange capacity	Titration value	pH	Base exchange capacity	Titration value
P.C.	*per cent*													
2	59.35	60.0	54.4	7.98	55.8	8.26	55.0	57.0	8.12	55.0	56.0	8.3	51.0	57.0
4	15.2	10.0	6.4	8.48	6.0	8.30	8.5	6.0	8.10	10.0	5.4	8.0	8.0	5.4
5	12.34	10.5	6.8	8.80	6.5	8.0	6.0	5.5	7.65	5.5	5.0	8.23	6.5	5.5
6	26.40	6.2	8.0	5.42	2.8	8.10	8.0	8.8	7.92	9.0	8.5	7.78	9.5	8.0
7	21.78	11.4	7.28	9.46	8.0	7.7	8.5	3.4	7.5	10.0	3.5	8.48	5.0	5.0
11	32.81	30.3	26.0	7.5	25.0	8.22	25.0	27.0	8.7	26.0	28.0	8.1	18.0	26.5
12	3.82	6.3	4.88	5.9	0.7	7.7	4.0	3.4	7.78	4.0	3.6	7.5	4.0	3.1
13	58.9	58.0	40.0	7.5	40.0	8.0	50.0	45.0	8.23	49.0	50.0	8.20	49.0	50.0
20	6.5	2.65	3.8	5.74	1.1	7.58	4.0	1.2	8.25	5.0	1.9	7.14	3.0	1.0
26	22.56	8.0	7.68	6.4	1.2	7.18	7.0	1.9	7.66	5.5	2.3	7.66	5.5	2.3

SOIL NUMBER	POTASSIUM CHLORIDE, LOW pH			POTASSIUM CHLORIDE, HIGH pH			POTASSIUM CARBONATE			KH_2PO_4 (SCHOFIELD)		
	pH	Base exchange capacity	Titration value	pH	Base exchange capacity	Titration value	pH	Base exchange capacity	Titration value	pH	Base exchange capacity	Titration value
P. C.												
2	8.85	52.0	59.0	9.0	55.0	59.5	10.23	88.0	84.0	6.28	16.3	46.0
4	8.58	8.0	6.0	9.0	10.0	7.0	10.85	23.0	15.5	7.16	3.4	4.0
5	8.98	6.1	6.5	9.08	9.5	7.0	10.87	18.0	16.0	7.08	2.6	4.0
6	5.12	2.7	0.5	9.26	13.0	13.0	10.4	23.0	21.0	6.8	2.4	5.0
7	9.0	7.8	6.5	9.1	10.0	6.5	10.97	14.0	19.0	7.0	1.0	1.9
11	9.02	28.2	28.6	9.7	32.0	30.0	10.7	46.0	36.0	6.65	10.4	10.0
12	6.1	1.0	0.8	8.6	7.0	4.5	10.66	10.0	10.8	6.9	0.8	1.8
13	8.44	49.0	53.0	10.0	68.0	71.0	10.38	88.0	84.0	6.16	15.0	23.0
20	6.1	2.7	0.3	9.35	3.5	3.3	10.66	15.0	7.2	7.0	0.2	0.8
26	7.75	1.0	2.5	8.76	9.0	6.0	10.82	14.0	13.6	6.94	1.8	1.8

acetate, and the remaining soil has a lower base-exchange capacity than the original soil. It is for this reason that Taylor and co-workers (10) found, by the ammonium acetate method, a lower base-exchange capacity than the total exchangeable bases in certain soils rich in organic matter.

COMPARISON OF DIFFERENT METHODS

The following methods were used:

Parker (4). The soil is treated with $Ba(OH)_2$ for 16–18 hours at room temperature, filtered, and leached with ammonium chloride followed by leaching with ethyl alcohol till free from chloride. The ammonia retained is determined by distillation with MgO.

Puri (5). (*a*) The soil is leached with *N* KCl, then with *N* $(NH_4)_2CO_3$. The filtrate is evaporated to dryness, and the alkali residue is found by titration. (*b*) The same as (*a*) except that 100 cc. 0.1 *N* KOH is added, and the suspension is shaken for 24 hours before KCl leaching.

Kelley (3). The soil, if acid, is shaken with 100 cc. *N* $Ba(OH)_2$ for 24 hours. It is then filtered and digested with 100 cc. *N* ammonium acetate for 8 hours at 70°C. followed by leaching with ammonium acetate until free from Ca. Excess of ammonium acetate is then removed with methyl alcohol, and the ammonia retained is determined by aeration after the addition of 5 per cent Na_2CO_3.

Chapman and Kelley (1). The same as the Kelley method except that digestion with normal ammonium acetate is continued for 15 hours.

Schofield (9). (*a*) The soil is shaken with K_2CO_3 solution, and the amount of alkali taken up is determined by titrating the filtrate. (*b*) The soil is shaken with KH_2PO_4 solution, and the amount removed by the soil is determined by taking the conductivity of the solution before and after shaking with the soil.

The number of methods could be increased by substituting one salt for another, but the methods outlined in the foregoing are sufficient to illustrate the general principles involved.

It is extremely difficult to ensure by any one method the bringing of all types of soils to the same pH value, but even if this were possible, the method would still remain far from satisfactory. The neutralization point of soil acidoids, like that of weak soluble acids, lies at a point 4 pH units higher than the pH value of the acidoid, which is not the same for all soils. By the very nature of things, it would be quite impossible to define the base-exchange capacity of soils with reference to any single pH value or any particular treatment. The most logical method of defining the acidoid equivalent of soils, popularly known as their absorption or base-exchange capacity, would be through their titration curves. The lack of attention so far paid to this fundamental property of soil acidoids is amazing.

The titration curve of a soil acidoid is not only reproducible but easily determined by the following technic. Twenty to thirty grams of soil is treated with enough acid to break up all the carbonates. The treated soil is transferred to a Büchner funnel and leached with 0.05 *N* HCl in 100-cc. lots until the filtrate is free from Ca ions. It is then leached with water until free from Cl ions, and finally with a few cubic centimeters of alcohol to facilitate drying and to prevent caking. The leaching is preferably done without suction, and the filter paper is attached to the funnel by running a little molten wax around the edges. The soil on drying is easily detached from the filter paper, and 2-gm. portions of the treated soil are shaken for 48 hours with 10 cc. of NaOH solution containing increasing amounts of alkali. The pH values are determined with the quinhydrone or the glass electrode. From the titration curve

thus obtained, the $T/2$ value (acidoid equivalent) is interpolated at a point 4 pH units higher than the initial pH. This value is more nearly fundamental and brings out the acidoid character of the soil colloids much better than is possible by any other arbitrarily fixed method of leaching.

It is a remarkable fact that the quinhydrone electrode can be used for pH values up to 9, and as will be seen from figure 1 the titration curves are almost identical to those obtained with the glass electrode. Although the newer types of glass electrodes are extremely simple to use, the entire outfit is costly. Some laboratories may prefer, therefore, to use the quinhydrone electrode, which is entirely satisfactory up to the pH range required for the titration curves of soils.

The results obtained with 10 soils by the use of different methods are given in table 2. The pH values at the end of leaching in every method was determined. The alkali equivalent corresponding to every pH value was interpolated from the titration curve, as was the $T/2$ value. The following conclusions are drawn from the results of this comparison:

The base-exchange capacity corresponding to any method depends on the final pH value obtained at the end of the leaching process and is approximately equivalent to the alkali required to bring the soil to that pH value.

The total exchangeable bases present in the soil as determined by single-treatment methods are in all cases approximately equivalent to the base-exchange capacity determined by neutral salt leaching. Slight differences may be due to the fact that bases originally present are different from those introduced by leaching with neutral salt.

The KCl method gives results similar to those obtained with ammonium chloride or acetate leaching but is very much simpler and quicker.

Schofield's K_2CO_3 method takes the soil to such a high pH value that the titration curves in that region are almost asymptotic when a slight difference in pH value can produce a large change in alkali equivalent. His KH_2PO_4 method is applicable only to acid soils and is therefore of restricted utility.

There is very good agreement between $T/2$ values and the titration values to initial pH with NaOH except in the case of acid soils. This would indicate that soils in nature, unless acidic, have a pH value that lies 4 pH units above their ultimate pH value. This is perfectly logical because any alkali over and above this point would be hydrolyzed and likely to be leached off.

The final pH of the soil after leaching with KCl is higher than that after leaching with NH_4Cl or ammonium acetate. This is due to the fact that ammonia is a very much weaker base than KOH and therefore leaves the soil at a lower pH.

It must be pointed out that the determination of equivalent point of weak acids by titration is valid only when the alkali used is strong. It is for this reason that the titration values with NaOH are recorded.

The fact that for most of the agricultural soils (having pH values above 7) the total exchangeable bases are equivalent to the base-exchange capacity as determined by leaching with a neutral salt is very significant and is confirmed from an examination of a number of soils of different types, the results of which are given in table 3. The base-exchange capacity was determined by the NaCl method previously described (5).

TABLE 3

Base-exchange capacity and total exchangeable bases of soils

SOIL NUMBER	CLAY (0.002 MM.)	pH	BASE-EXCHANGE CAPACITY BY NaCl METHOD	TOTAL EXCHANGEABLE BASES
P. C.	*per cent*		*m.e./100 gm.*	*m.e./100 gm.*
1	11.3	8.45	5.1	10.15
2	59.35	8.21	62.85	63.55
3	62.2	7.64	67.71	67.0
4	15.2	8.55	8.79	11.05
5	12.34	8.77	7.77	11.1
6	28.4	5.29	10.16	7.3
7	21.7	9.58	8.08	11.45
8	25.17	8.41	20.03	24.55
9	21.6	5.76	7.67	5.3
10	35.65	8.71	28.14	29.64
11	32.81	8.77	27.97	32.5
12	3.82	5.83	4.34	6.3
13	58.9	8.53	56.64	58.9
14	22.34	5.37	13.47	6.1
15	21.9	7.71	10.96	12.45
16	7.3	8.74	5.26	7.35
17	14.2	8.20	8.79	9.85
18	22.2	5.79	11.80	6.9
19	42.36	8.40	26.48	26.85
20	6.5	5.64	3.44	2.65
21	13.5	8.25	11.57	14.25
22	15.16	6.85	13.26	11.85
23	11.35	7.41	11.10	10.95
25	4.02	7.40	1.92	3.8
26	22.56	8.11	4.98	8.0
27	53.25	9.03	55.66	52.3
28	44.6	8.38	36.86	39.0
29	62.98	8.05	53.28	52.0
30	54.1	8.45	57.22	57.9
34	11.29	7.63	6.72	7.8
35	18.26	7.98	9.06	13.0
36	11.70	8.46	8.24	7.2
39	8.47	9.11	12.78	14.6
40	13.1	7.65	10.50	12.4
41	53.38	8.74	51.54	53.0
42	53.38	9.00	58.06	56.3
43	19.7	8.41	15.72	19.8
44	8.4	8.54	6.58	9.5
45	10.7	7.45	5.08	7.0
49	27.30	6.33	16.74	17.2
50	17.70	8.54	8.98	13.4
51	12.23	8.68	7.08	11.8
52	11.31	8.02	7.14	8.5
59	10.9	8.86	7.06	9.6

This point follows, as a matter of course, from the fact that the replacement of bases takes place in equivalent amount and unless the soil is acid the bases removed by leaching with a neutral salt (i.e., total bases) must be equivalent to the single base introduced. The important point about these studies, however, is that the total bases were determined by single-treatment methods developed by the senior author and described elsewhere (6, 7, 8).

Since these single-treatment methods give exchangeable bases (Ca, Na, K, and Mg) in a very much shorter time than any of the standard methods of determining base-exchange capacity, it does not seem worthwhile to determine the base-exchange capacity when the total exchangeable bases have been determined by single-treatment method.

The desirability of introducing a new terminology like the $T/2$ value or the base equivalent of the acidoid and of advocating the discarding of base-exchange capacity or their prototypes may be questioned. It must be emphasized, however, that these older terms are not only meaningless in so far as they refer to no uniform basis of comparison, but their continual use is likely to hinder the progress of soil science by perpetuating the use of methods that are essentially empirical. A number of terms in soil science are relics of the days when every reaction in soils was put down to "adsorption." With the advent of the chemical conception, these terms should either acquire a newer orientation or be discarded altogether.

SUMMARY

Methods of finding base-exchange capacity of soils have been critically reviewed. It is shown that different methods refer to different points on the titration curves of soils and, as such, are arbitrary and empirical. The only satisfactory method of defining the base-exchange properties of soils is through their titration curves, the determination of which is just as easy as is the finding of base-exchange capacity by any of the well-known methods.

REFERENCES

(1) CHAPMAN, H. D., AND KELLEY, W. P. 1930 The determination of replaceable bases and the base-exchange capacity of soils. *Soil Sci.* 30: 391–406.

(2) CSIKY, J. S. 1932 On the determination of the ion exchange capacity of soils. *Soil Sci.* 34: 269–279.

(3) KELLEY, W. P. 1929 The determination of the base exchange capacity of soils and a brief discussion of underlying principles. *Jour. Amer. Soc. Agron.* 21: 1021.

(4) PARKER, F. W. 1929 The determination of exchangeable hydrogen in soils. *Jour. Amer. Soc. Agron.* 21: 1030–1039.

(5) PURI, A. N. 1934 A new method of determining base exchange capacity of soils. *Soil Sci.* 37: 105–108.

(6) PURI, A. N. 1935 Estimation of replaceable Na and K, exchange capacity, and degree of alkalization in alkali soils by ammonium carbonate extraction. *Soil Sci.* 40: 249–253.

(7) PURI, A. N. 1935 A simple method of estimating exchangeable calcium and other bases in non-calcareous soils. *Soil Sci.* 40: 383–390.

(8) PURI, A. N. 1936 Estimating exchangeable calcium and other cations in soils. *Soil Sci.* 42: 47–59.

(9) SCHOFIELD, R. K. 1933 Rapid methods of examining soils. III. The use of dihydrogen potassium phosphate in studying the base exchange capacity. *Jour. Agr. Sci.* 23: 255–260.

(10) TAYLOR, E. M., ET AL. 1936 A study of the soils in the hill area of the Kulu Forest Division, Punjab. I. An investigation of the soil profile under deodar, spruce, blue-pine and chir. *Indian Forest. Rec.* (n. s. silviculture) 1 (2): 289–345.

BOOK REVIEW

Humus—Origin, Chemical Composition, and Importance in Nature. SELMAN A. WAKSMAN, Professor of Soil Microbiology, Rutgers University, and Microbiologist, New Jersey Agricultural Experiment Station. Second Edition. The Williams & Wilkins Co., Baltimore, 1938. Pp. xiv + 526, figs. 44, charts 3, tables 63. Price $6.50.

There is considerable significance in the fact that a second edition of this comprehensive treatise has appeared within the short period of two years from the time that the book was first published.[1] As it is neither a textbook nor a popularized discourse, there may have been reason to anticipate rather limited circulation of the volume. That this was not the case gives evidence of a widespread interest in the relationships of organic matter to soils and may be interpreted as a suggestion that there is growing realization of the great importance of organic matter in soil economy.

The volume has not been greatly changed from the first edition. The material is organized as before and, with few exceptions, has not been very much altered in scope. One new chapter has been added, that entitled "Humus and Soil Conservation" (8 pages), which appropriately gives recognition to a subject which has been gaining in interest and significance during recent years. Numerous changes, substitutions, and additions have been made throughout the book without appreciably altering its size. Reference has been made to a considerable amount of new material, as is evident from the fact that there were 1311 numbered references in edition one and 1608 in the new edition.

The author states in the preface:

> The recent publication of the Russian translation of this book served to direct the author's attention to the very extensive Russian literature, which was not given sufficient consideration, largely because of its inaccessibility. The attempt, made in this second edition, to correct somewhat this lack, was facilitated by the recent appearance of Professor Tiurin's book, "Soil Organic Matter." . . . The growing appreciation of the rôle of organic matter in soil conservation necessitated the addition of a new chapter and the considerable enlargement of another (stable manure, plant residues, green manures). Cognizance was also taken of the numerous recent papers on organic matter decomposition and on the functions of humus, no attempt being made, however, to cover this literature completely.

The author has once more performed a valuable service by providing the revised edition of *Humus*. Persons interested in the properties and utilization of soils, soil conservation, the microbial transformation of organic materials, and the nature and uses of peat and similar natural deposits of organic matter will find the volume a valuable source of useful information.

ROBERT L. STARKEY

[1] A review of the first edition was published in this journal, v. 41, p. 395-396, 1936.

CATION INTERCHANGE BETWEEN PLANT ROOTS AND SOIL COLLOIDS

H. JENNY AND R. OVERSTREET[1]

University of California[2]

Received for publication September 10, 1938

The present-day approach to the study of plant nutrition in soils centers around the *soil solution*. Investigators differ in their definitions of soil solution, but all postulate that a nutrient element must first enter the soil solution before it can be taken up by plant roots. Most definitions further assume that a nutrient ion in the soil solution is free to move. It is no longer a part of the solid phase, and it is not constrained by surface forces. In brief, the soil solution is essentially identical with the nutrient solution of the plant physiologist.

If plants are grown in mixtures of sand and Ca-clay, it is found that the roots accumulate Ca ions, whereas the clay particles gain in adsorbed hydrogen ions (4). From the viewpoint of the solution theory the reaction mechanism may be schematically formulated as follows:

$$\boxed{\text{Root}} \rightarrow \underbrace{m(H_2CO_3)}_{\text{In soil solution}} + \boxed{\text{Clay}}\begin{matrix}\text{Ca}\\ \text{Ca}\end{matrix} \rightarrow$$

$$\boxed{\text{Clay}}\begin{matrix}\text{H}\\ \text{H}\\ \text{Ca}\end{matrix} + \underbrace{Ca(HCO_3)_2 + (m-2)(H_2CO_3)}_{\text{In soil solution}} \qquad (A)$$

The plant roots excrete carbonic acid into the soil solution. The hydrogen ions, by means of ionic exchange, liberate calcium ions from the clay particles. The resulting calcium bicarbonate is a part of the soil solution, and Ca is now ready for intake by roots.

The most pertinent characteristic of equation *A* is the absence of any specification regarding the spatial distance between root and clay particles. As far as the general mechanism is concerned, it makes no difference whether root and clay particle are close together or far apart. Of course, the CO_2 concentration is likely to be highest in close proximity to the root surface, but this merely modifies the value of m in the equation and does not alter the fundamental nature of the process.

[1] The authors are indebted to D. R. Hoagland for his interest and valuable suggestions.

[2] Division of Plant Nutrition, College of Agriculture, Berkeley, California.

In this paper data are presented which indicate that the classical solution theories do not fully account for plant behavior in colloidal clay suspensions. It appears that the intimate contact between root and clay is far more significant than heretofore has been realized.

CONTACT EXCHANGE

The adsorbed cations on the surface of colloidal clay particles are not held rigidly. As a result of thermal agitation they oscillate and, at times, may be at considerable distances from the surface; but they remain in the field of force emanating from the colloid. Although the ions are surrounded by water molecules, they are not in solution in the sense that they can diffuse freely. The cations are under conditions of constraint and follow closely the movements of the colloidal particle to which they are attached.

The surface cations may be released by exchange and then become an

FIG. 1. SCHEMATIC REPRESENTATION OF BASE EXCHANGE OR IONIC EXCHANGE

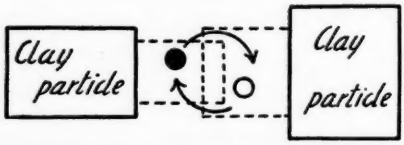


FIG. 2. SCHEMATIC REPRESENTATION OF CONTACT EXCHANGE

integral part of the intermicellar liquid or soil solution. Exchange may occur when a replacing cation enters the oscillation space of the adsorbed cation (3), as illustrated in figure 1. This process, known as base exchange or ionic exchange, forms the basis for the mechanism represented by equation *A*.

Conceivably, ionic exchange might also occur if two oscillation spaces overlap. This process could take place for neighboring ions on the same surface, and also between ions belonging to different particles, provided that the micelles approach so closely that the oscillation spaces interpenetrate. Figure 2 schematically illustrates this second type of exchange, which might be designated as *contact exchange.*

An important feature of this mechanism is the fact that the reaction, as such, is independent of the nature of the "soil solution." To use a more picturesque expression, one might say that the ions do not enter the soil solution *per se*, but, in the moment of contact, they jump directly from one particle to another. This theory leads to a new concept of the mechanism of migration

of ions in gel systems and colloidal suspensions. The ions diffuse along the surfaces of the colloidal particles rather than in the pores or intermicellar liquid. Preliminary evidence of this surface migration of ions has been obtained in the senior writer's laboratory by Eric Winters.[3]

CONTACT EFFECT BETWEEN ROOTS AND SOILS

When plant roots are immersed in clay suspensions the root surfaces are subjected to heavy bombardments by colloidal particles (Brownian movement), and some sort of interaction is to be expected. If negative clay ultramicrons collide with negative root surfaces, the phenomenon of contact exchange might enter into play. Nutrient cations adsorbed on clay particles could thus become attached to plant roots directly rather than by way of the soil solution. Since contact exchange involves a mutual transfer of ions, it follows that for every cation gained by the root, an equivalent number of ions

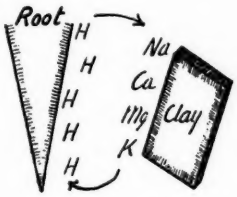


FIG. 3

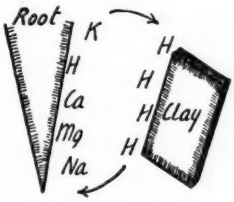


FIG. 4

FIG. 3. SCHEMATIC REPRESENTATION OF CONTACT INTAKE
FIG. 4. SCHEMATIC REPRESENTATION OF CONTACT DEPLETION

must leave the root surface. For this reason we must distinguish between *contact intake* and *contact depletion*, as illustrated in figures 3 and 4. In contact intake the hydrogen ions on the root surface exchange for nutrient ions on the clay. The root system gains bases, whereas the clay particle becomes enriched in H ions. In contact depletion the roots are surrounded by H-clay particles, and, upon contact, any nutrient ions that might be on the root surface may be transferred to the clay surface. In other words, roots immersed in a suspension of H-clay should experience a loss of nutrient cations.

In the present study an attempt is made to investigate experimentally the effect of colloidal clay suspensions on the mineral composition of plant roots. Because of the possible existence of a high CO_2 gradient in the immediate vicinity of the root, the phenomenon of contact feeding may be accompanied by solution processes such as those indicated by equation *A*. For this reason it is less easily observed experimentally than the process of contact depletion. Theoretically, the latter is particularly striking, since the soil solution theories do not operate with the concept of root depletion. Consequently, all our

[3] Thesis, University of Illinois, 1938.

studies concentrate on contact depletion of roots. In this paper, data on the element potassium will be presented. The behavior of other cations will be treated in subsequent publications.

EXPERIMENTAL TECHNIC

All experiments were conducted with barley plants of the Sacramento variety. They were grown according to the method of Hoagland and Broyer (2). The plants are characterized by a relatively low potassium content. The roots readily accumulate potassium from highly dilute solutions and thus possess an inherent tendency to resist depletion of K to the surrounding solution. The seeds were germinated in special chambers and then transplanted to shallow pans. In each pan were 168 plants held by 24 corks. The pans were filled with 3,800 cc. of ½ Hoagland solution. The standard solution of full strength has the following composition:

KNO_3	0.005*M*
$Ca(NO_3)_2$	0.005*M*
$MgSO_4$	0.002*M*
KH_2PO_4	0.001*M*

Iron, as iron tartrate (0.5 per cent solution), was added twice a week, in the proportion of 1 cc. per liter. After the initial filling of the pans, no new nutrient solution was added, but the volume was maintained by the addition of distilled water daily. After 3 weeks the barley plants, which had grown to about 18 inches in length, showed definite signs of starvation. At this stage they were used for experimentation.

In order to eliminate the complications of root and shoot relationships, a number of trials were run with roots only. As Hoagland and Broyer have shown, these excised roots, under proper conditions of aeration, are in an active state of metabolism and exhibit pronounced salt accumulation over a period of about 20 hours. In all our experiments the period was limited to 10 hours. The tests were conducted with the roots of 168 plants or, in a few cases, of 84 plants. We selected the following types of root studies:

Excised roots (method of Hoagland and Broyer). The roots were cut off below the seed hulls and after being washed in distilled water were immersed in the desired solutions, which were contained in glass jars of 3-liter capacity. In favorable seasons one pan usually yields about 100 gm. of fresh root material (weight determined after centrifuging) which may be conveniently handled in the 3-liter glass jar. The containers were placed in a thermostat and a continuous stream of CO_2-free air was passed through the system. After completion of the test period, the roots were again washed in distilled water, dried at 60°C., and, after being ground, subjected to chemical analysis.

Decapitated plants. To avoid possible errors caused by exudations from the cut root ends, a second method was employed, in which the tops were cut off ¼ inch above the corks. These decapitated plants were left in the corks, and only the roots were dipped into the desired solution, which was placed in

shallow glass trays. These systems were not artificially aerated. The protruding cut ends were continuously watched, and all exudates were carefully removed with filter paper. At the end of the experiment, the roots were cut off below the seed hull, and the roots, the remaining basal parts of the shoots, and the exudates were analyzed separately. This procedure is more cumbersome than the first method, in which the roots were excised, but permits a more rigid examination of the outgo and intake of ions through the root surface.

EXPERIMENTAL DATA

The presentation of the experimental data is organized under the following three headings: behavior of roots in distilled water; behavior of roots in salt solutions; behavior of roots in clay suspensions.

TABLE 1

Behavior of excised roots in distilled water

(Potassium content of roots, milliequivalents per 100 gm. oven-dry material)

DATE	DRY WEIGHT OF ROOTS PER PAN	K CONTENT OF ROOTS		DIFFERENCE	REMARKS	LABORATORY NUMBER
		No treatment	In H_2O			
	gm.	*m.e.*	*m.e.*	*per cent*		
Dec. 10, '37	5.29	41.8	....	.....		1
"	5.16	....	45.9	+9.8	In 3 liters H_2O	2
Jan. 7, '38	3.66	43.1	....	.....		3
"	3.76	....	42.5	−1.4	In 3 liters H_2O	4
"	3.81	....	42.3	−1.9	"	5
"	3.99	....	42.2	−2.1	In 100 gal. H_2O	6
"	3.58	....	41.4	−4.0	"	7
Apr. 1, '38	7.96	32.2	....	.....		8
"	8.12	....	32.0	−0.6	In 3 liters H_2O	9
"	7.00	....	31.2	−3.1	"	10
May 20, '38	7.54	26.0	....	.....		11
"	....	....	25.4	−2.5	"	12

Behavior of excised roots in distilled water

At the outset it was necessary to determine the loss of potassium when the excised roots are immersed in distilled water and aerated to insure optimum conditions for metabolism. The data are assembled in table 1. The column headed "No treatment" refers to roots which were briefly rinsed in distilled water and then immediately dried. All other roots were kept in distilled water for 10 hours.

In general, the results are very consistent. In distilled water (3 liters) the roots suffer a loss of K of only a few per cent, the maximum being 3.1 per cent. This is probably within experimental error. Special attention should be directed to experiments 6 and 7. Here the roots were continuously leached with distilled water at the rate of 10 gallons an hour; in other words,

about 380 liters of water passed around the root systems. Yet, the loss of K is of the same order of magnitude (2.1–4.0 per cent) as that for the 3-liter equilibrium sets. In one case, experiment 2, the roots gained K, which must be attributed to impurities.

To summarize, we may conclude that *the low salt roots under investigation have a pronounced capacity to retain potassium ions against distilled water.*

It will be of interest to compare the foregoing results with those from plants of high salt content. These were also grown in Hoagland solutions, but unlike the low salt systems, the nutrient solutions were renewed every other day. The "No treatment" roots weighed 4.19 gm. and contained 148.4 m.e. K per 100 gm., on the oven-dry basis; whereas the corresponding values for the leached root were 3.75 gm. and 136.2 m.e. K. The K losses produced by 3 liters of distilled water amounted to 12.2. m.e., or 8.2 per cent. It is evident that the losses of K from high salt plants assume very substantial magnitudes.

Behavior of excised roots in true solutions

Since clays carry adsorbed cations, particularly Ca, Na, H, and NH_4, the question naturally arises whether these ions *per se* exert some specific influence on the condition of the root in relation to its retaining power for potassium. Accordingly, excised root systems of low salt plants were submerged for 10 hours in 3 liters of the following solutions: $CaCl_2$, HCl, $NaHCO_3$, NH_4HCO_3, and KCl. The K content of the roots thus treated was then compared with that of the same roots in distilled water. The results are given in table 2.

It will be noted that the roots are extremely active in absorbing K from KCl: during 10 hours the potassium content of the roots was augmented by over 100 per cent. As to *the remaining salt solutions, in no case is there a significant loss of K from the root systems.* The gain in the case of $NaHCO_3$ is probably caused by impurities from the salt or equipment (aerators). Hydrochloric acid occupies an exceptional position. The solution was renewed every hour in order to maintain an acid reaction of approximately pH 4.1. The roots lost 5 per cent of their potassium content, an amount which probably is statistically significant. This result will be of particular interest in the interpretation of the behavior of H-clays, which is next considered.

Behavior of roots in clay suspensions

In this phase of the study the single-salt solutions are replaced by clay suspensions, that is, by colloidal solutions of clays which carry but one type of adsorbed cation; namely, H, or Na, or K, or NH_4, or Ca, or combinations of these ions. These clays are so-called bentonites which consist of the common clay mineral montmorillonite. They possess a cation adsorption capacity of about 100 m.e. per 100 gm. of oven-dry clay (ammonium acetate method). Prior to experimentation the clays were electrodialyzed for 5 to 6 months, during which process they were converted into H-bentonites. The basic clays were obtained by adding hydroxides [NaOH, KOH, NH_4OH, $Ca(OH)_2$] in

amounts equivalent to the exchange capacity. The reaction may be represented as follows:

$$\left[\text{Clay}\right]\begin{matrix}H\\H\\H\end{matrix} + 3NaOH \rightleftharpoons \left[\text{Clay}\right]\begin{matrix}Na\\Na\\Na\end{matrix} + 3HOH \qquad (B)$$

These colloidal suspensions are very stable.

As far as could be determined the clay particles do not permanently adhere to the root surfaces. Merely rinsing with distilled water gives clean roots. This is to be expected on theoretical grounds, as both root and clay are electrically negative, and, as is well known, colloidal particles of like charge repel each other.

TABLE 2

Behavior of excised roots in electrolyte solutions

(Potassium content of roots, milliequivalents per 100 gm. oven-dry material)

ELECTROLYTE	CONCENTRATION	K CONTENT OF ROOTS	DIFFERENCE DUE TO SOLUTION	pH OF SOLUTION		DATE	LABORATORY NUMBER
				Initial	Final		
	m.e./l.	*m.e.*	*per cent*				
H_2O	....	61.3	.....	....	....	July 21, '37	14
KCl	5.0	126.5	+106.2	6.65	6.14	"	15
H_2O	....	27.0	.....	5.65	5.65	May 19, '38	16
NaCl	9.0	25.9	−4.1	5.60	5.30	"	17
NaCl	19.0	26.4	−2.2	5.15	5.00	"	18
H_2O	....	31.2	.....	....	....	Apr. 3, '38	10
$CaCl_2$	3.0	31.6	+1.4	....	....	"	19
HCl	0.1	29.6	−5.1	4.10	4.10	Apr. 1, '38	20
H_2O	....	25.4	.....	5.55	5.75	May 20, '38	12
$NaHCO_3$	5.0	27.5	+8.3	8.30	7.58	"	21
NH_4HCO_3	5.0	25.8	+1.6	7.90	7.18	"	22

Excised roots in unsaturated clays. The unsaturated clay systems studied are H-bentonites, the outer electric double layers of which contain only H ions. The effects of these systems on the roots are extreme. The clays virtually deprive the roots of their entire potassium content. At the same time the roots experience a loss in turgor.

In one instance, H-bentonite was dried, before being used, and then resuspended. This coarse, flaky suspension contained larger but fewer particles, yet the adsorption capacity per unit weight of clay remained the same. This relatively unstable suspension pulled out smaller amounts of K (26 per cent) in spite of the fact that 30 per cent more clay was added to the roots. This behavior is in line with the idea of contact phenomena, since fewer collisions between root surfaces and clay particles occur in the coarser systems.

One might contend that these findings do not prove contact effects, but are merely the consequence of the high acidity of the bentonite suspensions.

Indeed, from table 3 it is seen that the pH values of the sols are low, varying between 2.90 and 3.52. It should be kept in mind, however, that these pH values refer to the acidity of the entire suspension, that is to say, the glass electrode is affected by such H ions as are a part of the electric double layer which surrounds the particles (suspension effect). The hydrogen-ion concentration of the intermicellar liquid, the so-called soil solution proper, is much less, namely, pH 4.1 as measured by the collodion bag technic. Since HCl with a pH of 4.1 (see table 2) has but little effect on the root system, it is fair to assume that we are actually dealing with some sort of contact phenomenon,

TABLE 3

Properties of bentonite suspensions

Clays used for the excised root experiments reported in table 4; total volume of suspensions, 3,000 cc.

SYSTEM	CONCENTRATION OF CLAY	EXCHANGEABLE CATIONS PER LITER			pH OF SUSPENSION		LABORATORY NUMBER
		H	K	Ca	Initial	Final	
	per cent	*m.e.*	*m.e.*	*m.e.*			
Unsaturated clays:							
H-clay, sol	3.97	39.7	0	0	3.05	2.90	23
H-clay, flakes	5.17	51.7	0	0	3.70	3.40	24
H-clay, sol	1.00	10.0	0	0	3.25	3.52	25
Partially saturated clays:							
K-H-clay, sol	0.83	6.6	1.7	0	4.90	4.40	26
K-H-clay, flakes	1.16	9.2	2.4	0	5.10	4.30	27
Ca-H-clay, sol	0.48	1.9	0	2.9	5.65	5.25	28
Ca-H-clay, sol	2.10	10.5	0	10.5	5.10	5.05	29
Ca-H-clay, flakes	3.50	17.5	0	17.5	5.22	4.95	30
		Na	NH_4				
Saturated clays:							
Ca-clay, sol	0.94	0	0	6.8	7.37	6.43	31
Na-clay, sol	0.33	3.3	0	0	7.60	4.70	56
NH_4-clay, sol	0.33	0	3.3	0	7.50	5.14	55

perhaps a contact acidity effect. When a clay particle with its ion swarm comes into close vicinity of the root surface, the latter is temporarily subjected to a localized high concentration of H ions which affects the root in such a manner that it gives off potassium.

Excised roots in partially saturated clays. All bentonites used in the study with partially saturated clays have a cation adsorption capacity of 100 m.e. per 100 gm. of dry material. In the case of the electrodialyzed systems, the hydrogen ions are the only cations on the clay surfaces. If bases [KOH, $Ca(OH_2)$] are added to the H-clays in amounts which are below the value of

the saturation capacity, so-called partially saturated bentonites are obtained. The double layer consists of H ions as well as other cations (K, Ca).

The results obtained with partially saturated clays are shown in table 4. Regarding the low salt plants, an interesting feature is found by comparing the two sols: K-H-bentonite and Ca-H-bentonite. From K-H-clay the roots accumulate K and increase their K content by 28.5 per cent. At the same time they lose Ca ions, to the extent of 22.2 per cent. From Ca-H-clay the roots

TABLE 4

*Behavior of excised roots in bentonite suspensions**

(Potassium content of roots, m.e. per 100 gm. oven-dry material)

SYSTEMS IN WHICH ROOTS WERE SUSPENDED FOR 10 HOURS	CLAY CONCENTRATION	POTASSIUM		DATE	LABORATORY NUMBER
		In roots	Difference due to colloid		
	per cent	*m.e.*	*per cent*		
Unsaturated clays:					
H_2O	....	45.9	.....	Dec. 10, '37	2
H-clay, sol	3.97	4.7	−89.8	"	23
H-clay, flakes	5.17	34.3	−26.0	"	24
H_2O	....	42.0	.....	Jan. 7, '38	32
H-clay, sol	1.00	14.4	−66.0	"	25
Partially saturated clays:					
H_2O	....	45.9	.....	Dec. 10, '37	2
K-H-clay, sol	0.83	59.0	+28.5	"	26
K-H-clay, flakes	1.16	75.6	+64.6	"	27
Ca-H-clay, sol	0.48	37.0	−19.4	"	28
H_2O	....	136.2	.....	Dec. 8, '37	33
Ca-H-clay, sol	2.10	114.5	−15.9	"	29
Ca-H-clay, flakes	3.50	125.0	−8.2	"	30
Saturated clays:					
H_2O	....	40.0	.....	Oct. 25, '38	53
H_2O	....	39.0	.....	"	54
NH_4-clay, sol	0.33	26.7	−32.4	"	55
Na-clay, sol	0.33	34.1	−13.7	"	56
H_2O	....	29.5	.....	Jun. 3, '38	34
Ca-clay, sol	0.94	27.8	−5.8	"	31

* Compare with table 3.

take up Ca ions (6.4 per cent) but experience a loss of K ions amounting to 19.4 per cent. Thus, we observe a differential intake and outgo of nutrient cations. The pH values (see table 3) are not low, especially in the Ca-H-system (pH 5.65); therefore the acidity of the suspension, as such, can hardly account for the loss of cations.

High salt roots immersed in Ca-H-bentonites suffer corresponding losses of K. The sol depleted the K content of the roots by 16 per cent; the reduction caused by the coarse system (flakes) was 8 per cent.

Excised roots in saturated clays. When a suspension of electrodialyzed bentonite is treated with an amount of base [NaOH, NH_4OH, or $Ca(OH)_2$] which is equivalent to the exchange capacity, a saturated system results. Suspensions of this type were prepared for Na, NH_4, and Ca (table 3). Low salt, excised barley roots were immersed in these systems for 10 hours and subsequently analyzed for potassium. The analyses, together with the results for corresponding roots immersed in water, are recorded in table 4. Considerable amounts of K were removed from the roots, especially with the NH_4-system.

Experiments with decapitated plants. The results obtained with the excised root technic seem to demonstrate contact effects. The possibility that a purely physiological feature is involved is not, however, completely ruled out. The clays may merely adsorb the ions exuded from the cut portions of the root and in this manner bring about enhanced bleeding which depletes the roots in nutrient ions.

The decapitated root technic was devised to eliminate the possible contribu-

TABLE 5

Properties of bentonite suspensions used in decapitated plant systems

	CLAY CONCENTRATIONS	pH OF SUSPENSIONS		LABORATORY NUMBER
		Initial	Final	
	per cent			
H-bentonite	0.42	3.95	....	35
H-bentonite	0.53	3.85	4.07	36
Ca-bentonite	0.31	7.40	6.37	37
Na-bentonite	0.48	7.35	6.70	38
NH_4-bentonite	0.41	7.25	....	39

tions of enhanced bleeding. As described in the section on experimental technic, the tops are cut off at a considerable distance above the root and the exudates are collected separately. In this manner it is possible to determine accurately the direct loss of ions to the solution. Because of the involved experimental technic, the studies were limited to comparisons between water systems and clay systems. The latter were monosystems, that is, only one kind of adsorbed cations occupied the clay surfaces (H, Na, NH_4, Ca). The properties of the bentonite clays used are reported in table 5. The trials were run for 10 hours, and the clay suspensions were renewed every 2 hours, except in the case of H-bentonite (no. 35), which was changed every hour.

Table 6 represents a summary of the bleeding data. For all ten systems studied the exudation of K is minute. On the basis of root analysis these exudation losses are much smaller than the experimental errors of K determinations. We may safely conclude that if these decapitated plant systems show significant losses of K in clay suspensions, the nutrient ion must have left those root portions which were immersed in the solutions.

The fact that these losses are very significant is evident from table 7. Up to one third of the potassium content of the root is given off to the colloidal solutions, which, in general, substantiates the results obtained with the excised root technic. The various bentonites may be arranged in a lyotropic series of the following form:

$$NH_4\text{-clay} > \text{H-clay} > \text{Na-clay} > \text{Ca-clay}.$$

Ammonium clay is most active, whereas Ca-clay in the concentrations used does not greatly affect the root system. That some sort of an ion exchange between root and clay is involved is shown by the simultaneous intake of the clay cations by the roots. The roots immersed in Ca-clay gained 24.2 per cent Ca; the roots of the Na-bentonite system increased their Na content by 149

TABLE 6

Summary of bleeding data

(m.e. K exuded on basis of 100 gm. oven-dry roots + basal parts of shoots during 10 hours)

SYSTEM	DATE	POTASSIUM EXUDED		LABORATORY NUMBER
			Per cent of total K	
		m.e.		
H_2O	Mar. 17, 1938	0.113	0.29	40
H-bentonite	"	1.30	3.33	35
H_2O	Mar. 30, 1938	0.028	0.08	41
H-bentonite	"	0.021	0.06	36
H_2O	"	0.051	0.15	42
Ca-bentonite	"	0.028	0.08	37
H_2O	Apr. 19, 1938	0.033	0.08	43
Na-bentonite	"	0.018	0.05	38
H_2O	May 17, 1938	0.002	0.006	44
NH_4-bentonite	"	0.060	0.19	39

per cent; and the NH_4-clay roots augmented their nitrogen content by 27.8 per cent. Similar relationships prevail in the basal parts of the shoots, except for Ca which apparently did not move upward.

A further point of interest is revealed by the pH measurements (table 5). The Ca-, Na-, and NH_4-bentonites used were saturated clays, and the initial pH of the suspensions was considerably above 7. Nevertheless, the Na- and NH_4-clays removed substantial amounts of K from the roots (table 6), which clearly shows that acidity cannot be the major cause of the observed contact effects.

After the experiment the clay suspensions were slightly acid (table 5), which suggests that the roots also have given off H ions to the clay particles. This confirms data for soybeans previously reported (4).

Experiments with entire plants and H-bentonites. In view of the very significant losses obtained in root experiments, a study of entire plants appeared

feasible. In these studies the plants were removed from the growth pans, their roots rinsed in distilled water, and transferred to glass pans which contained 1,800 cc. of distilled water or clay suspension. The bentonite sols were renewed every hour during the 10-hour period of experimentation.

A first experiment was performed on February 14, 1938. The concentration of the H-sol was 0.178 per cent, and the initial pH value was 4.30. After the experiment the pH had increased to 4.45. A second experiment was conducted on February 24, 1938. The concentration of this H-bentonite was 0.16 per cent. The initial and final pH values were 4.40 and 4.33 respectively.

The observations (table 8) are in full accord with the previous experiments. In both trials the H-bentonite, which was but moderately acid, lowered the

TABLE 7

Behavior of decapitated plants in water and clay suspensions

(Potassium content of roots and basal parts of shoots, m.e. per 100 gm. oven-dry material)

SYSTEM	POTASSIUM IN ROOTS*		POTASSIUM IN BASAL PARTS OF SHOOTS†		DATE (1938)	LABORATORY NUMBER
		Difference due to clay		Difference due to clay		
	m.e.	*per cent*	*m.e.*	*per cent*		
H_2O	40.5	.....	36.6	.....	Mar. 17	40
H-bentonite	31.4	−22.2	33.0	−9.8	"	35
H_2O	34.6	.....	37.1	.....	Mar. 30	41
H-bentonite	25.3	−26.8	35.9	−3.2	"	36
H_2O	33.3	.....	37.3	.....	"	42
Ca-bentonite	33.0	−0.91	36.1	−3.2	"	37
H_2O	29.8	.....	39.9	.....	Apr. 19	43
Na-bentonite	33.9	−14.8	37.7	−5.5	"	38
H_2O	34.4	.....	28.9	.....	May 17	44
NH_4-bentonite	23.2	−32.6	31.1	+7.6	"	39

* The average oven-dry weight of the roots is 7.53 gm.

† The average oven-dry weight of the basal parts of the shoots is 4.89 gm.

K content of the roots by approximately 20 per cent. The shoots of the first experiment did not vary in K; those of the second experiment experienced a loss of 6.8 per cent. This latter figure may not be significant, as the variability of the tops is much greater than that of the roots.

Permanent injury test. In consideration of the heavy nutrient losses of the plants, the question naturally arises as to whether the H-clays have permanently injured the root systems. Injury may either precede the outgo of K or be the consequence of the depletion of nutrient ions. Of the aforementioned two H-bentonites, the second served as a means to answer the question of contact injury. The water and H-bentonite test corresponding to Laboratory Number 47/48 was run in duplicate, and, following the ordinary period of 10 hours, one of the two water sets and one of the two bentonite sets

were transferred to a full Hoagland solution and kept there over night (12 hours). The analytical results are shown in table 8. Both systems have vigorously accumulated K ions and more than doubled the K content of the roots. Copious amounts of K moved into the shoots. Of particular significance is the fact that the plants which previously lost K to the clay suspensions were more active absorbers than those which had been kept in water, and consequently after 12 hours the K content of both sets became nearly alike. Thus, we may safely conclude that any contact injury which might have occurred in this experiment must have been brief and reversible, and did not permanently reduce the K-absorbing capacity of the roots. Instances of extreme losses of potassium (table 4), however, are most likely accompanied by permanent injury to the roots.

TABLE 8

Behavior of entire plants in H-bentonite suspensions

(Potassium content of roots and of shoots, m.e. per 100 gm. oven-dry material)

SYSTEM	ROOTS		DIFFERENCE	SHOOTS		DIFFERENCE	LABORATORY NUMBER
	Oven-dry weight	K content		Oven-dry weight	K content		
	gm.	*m.e.*	*per cent*	*gm.*	*m.e.*	*per cent*	
H_2O	4.62	46.1	.....	15.58	68.2	.....	45
H-bentonite	4.34	35.9	−22.1	15.68	68.2	0.0	46
H_2O	5.52	33.5	.....	17.90	55.6	.....	47
H-bentonite	5.74	26.8	−20.0	19.68	51.8	−6.8	48
		Composition of plants after treatment with Hoagland solution					
H_2O	5.04	68.7	+105	18.28	86.5	+55.6	49
H-bentonite	5.40	67.7	+153	20.16	81.6	+57.5	50

Some crucial experiments. The comparisons between untreated roots and those kept in distilled water (table 1) indicate a possible small outgo of K into water. If the soil colloids continuously adsorbed these excreted cations and removed them from solution there would conceivably be established a one-directional flow of K which might gradually deplete the root system.

Although the experiments on continuous leaching with 100 gallons of distilled water speak against such a mechanism, it seemed desirable to check the possibilities in some other manner. A *membrane experiment* was devised in which roots and clay were separated by a semipermeable membrane which afforded easy passage for cations but retained colloidal clay particles.

The excised roots were placed inside a membrane bag (Visking Casing) which had relatively large pores. The roots inside the bag were aerated as usual. The sack was immersed in a 0.82 per cent suspension of H-bentonite

which had a pH of 3.58. In a parallel experiment a second bag containing roots was placed in distilled water. All other conditions were the same. We have thus the following two comparable systems:

$$\text{No. 51: Roots} + H_2O \mid \text{Membrane} \mid \text{H-bentonite}$$

$$\text{No. 9: Roots} + H_2O \mid \text{Membrane} \mid H_2O$$

After the usual period of 10 hours the roots kept in water contained 32.0 m.e. K per 100 gm. of root, whereas the bentonite roots had 35.3 m.e./100 gm. In the absence of the membrane a loss of over 50 per cent K could have been anticipated; in the presence of the membrane a slight gain is found. In other words, lack of contact between root and clay failed to lower the K content of the roots.

Another means of testing the contact theory is afforded by the use of *positive colloids*. The barley roots possess a definite cation adsorption capacity of 11 m.e. per 100 gm. dry material, and the root surface is negatively charged, as indicated by streaming potentials. The bentonites also are electrically negative. On theoretical grounds, contact jumping of cations should occur only between surfaces of like sign, as is the case with roots and bentonite clays. Roots in contact with positive colloids would not be expected to give off K by contact exchange. Accordingly, excised roots were placed in a suspension of positive iron hydroxide sol which had an anion exchange capacity of 60 m.e. per 100 gm. colloid. It had no cation adsorption capacity. The sol was electrodialyzed for several days, during which the pH dropped to 4.50 (glass electrode). The concentration of the suspension used was 0.33 per cent.

Since root and iron sol are oppositely charged, mutual flocculation might be expected to occur. In consequence, some K might be set free according to the following equation:

$$\left[\text{Root}\left|\begin{matrix}K\\+\\H\end{matrix}\right.\begin{matrix}HO\\\\HO\end{matrix}\left|\text{Fe(OH)}_3\right.\right] = \left[\text{Root}\left|\text{Fe(OH)}_3\right.\right] + KOH + HOH \quad (C)$$

The experiment was carried out in accordance with the standard excised root technic previously described and was supplemented by a water check (experiments 52 and 10). The roots which remained in distilled water for 10 hours (experiment 10) had a potassium content of 31.2 m.e. per 100 gm. oven-dry material. The corresponding figure for the iron sol roots was 30.4 m.e. per 100 gm., which represents a negligible reduction of 4.4 per cent. Although the iron hydroxide sol partially coated the root surface, it did not stop the free passage of ions, as shown by a separate experiment in which roots covered with $Fe(OH)_3$ freely accumulated K and Br ions from a solution of KBr.

DISCUSSION

In 1928 Kelley (5) reported observations on the growth of barley plants in a carbonate-free soil which was made extremely toxic to roots by replace-

ment of the calcium with sodium. Investigations showed that the soil solution was entirely nontoxic. Kelley concludes,

> I am inclined to believe that the injury to the plant was produced through the pronounced avidity of the sodium exchange complex for calcium. The germinative seedlings contain a small amount of calcium derived from seeds. This calcium upon diffusing into the developing root is possibly absorbed by the sodium zeolites of the soil. The result is that the growing seedling is not only unable to secure calcium from the soil but the seedling is actually robbed, so to speak, of a portion of calcium that is supplied by the mother seed.

Jenny and Cowan (4) studied the behavior of soybean plants in Ca-clay systems. As the plants consumed Ca ions, the clay particles gained H ions. Data indicated that occasionally the roots transferred also mineral cations to the clay suspensions. Albrecht (1) grew soybean plants in clay systems of various degrees of saturation, e.g., Ca-H-clays. He observed that "colloidal clay deficient in nutrient cations has even taken these from the growing plant which finally contained less of these than were present in the seed at the outset." Lundegårdh (6), in his book on mineral nutrition of plants, states that soil colloids play only a passive rôle in the nutrient absorption by plants. He admits, however, that a certain competition between plant colloids and soil colloids is likely to occur which might lead to an occasional migration of nutrient ions from the root into the surrounding medium.

Our numerous experiments, which comprise a variety of technics, yield one particularly consistent result: dilute clay suspensions carrying adsorbed Na, NH_4, or H ions pull out considerable quantities of potassium ions from normal, low salt roots. Furthermore, this effect is not observed, or only to a slight extent, with corresponding salt solutions. Evidently some sort of contact phenomenon must be involved. When certain types of clay particles approach or touch the root surfaces the latter experience a loss in nutrient cations.

Regarding the mechanism of this contact effect, the situation is less definite. Although all results are in harmony with the theory of contact exchange, as formulated at the beginning of this paper, the possibility remains that some other explanation might equally well account for the facts observed. Further work needs to be done to establish the "truth" of the contact exchange theory.

SUMMARY

Low salt barley roots tenaciously retain their potassium against distilled water over a period of at least 10 hours.

Salt solutions of moderate concentrations do not appreciably influence the potassium level of the roots investigated.

Dilute clay suspensions carrying adsorbed Na, NH_4, or H ions pull out potassium from normal low salt roots. During the brief interval of 10 hours, the potassium level of the root may be reduced 15 to 90 per cent, depending on clay concentration, particle size, and the nature of the adsorbed cation. Ca-bentonites, in the concentrations used, do not greatly affect the potassium status of the roots.

If the roots and the clay particles are separated by a semipermeable membrane, the aforementioned effects are not observed. The effects are also absent if positive iron hydroxide sols are substituted for the negative clay sols.

It is concluded that these experiments reveal the existence of a contact phenomenon between plant roots and clay particles.

A theory of contact exchange is proposed according to which cations may transfer from clay to root and vice versa without entering the soil solution.

REFERENCES

(1) ALBRECHT, W. A., AND MCCALLA, T. M. 1938 The colloidal clay fraction of soil as a cultural medium. *Amer. Jour. Bot.* 25: 403–407.

(2) HOAGLAND, D. R., AND BROYER, T. C. 1936 General nature of the process of salt accumulation by roots with description of experimental methods. *Plant Physiol.* 11: 471–507.

(3) JENNY, H. 1936 Simple kinetic theory of ionic exchange. *Jour. Phys. Chem.* 40: 501–517.

(4) JENNY, H., AND COWAN, E. W. 1933 The utilization of adsorbed ions by plants. *Science* 77: 394–396.

(5) KELLEY, W. P. 1928 A general discussion of the chemical and physical properties of alkali soils. *Proc. First Internatl. Cong. Soil Sci.* 4: 483–489.

(6) LUNDEGÅRDH, H. 1932 Die Nährstoffaufnahme der Pflanze. Jena.

BASE CONTENT OF CORN PLANTS AS INFLUENCED BY pH OF SUBSTRATE AND FORM OF NITROGEN SUPPLY[1]

C. H. WADLEIGH AND J. W. SHIVE

New Jersey Agricultural Experiment Station

Received for publication September 7, 1938

In a previous paper (21) the marked influence of the presence of ammonium nitrogen in the substrate upon the appearance of the plant in general and upon development of chlorosis in particular was indicated. It was pointed out, in connection with the pronounced influence of the ammonium ion in the substrate upon the vigorously healthy external appearance, that there were marked differences in certain components of the expressed sap of the plants of two series; plants of one series receiving nitrate nitrogen only, and those of the other receiving both nitrate and ammonium nitrogen. For example, it was noted that for any given pH level of the nutrient solution, the pH of the expressed sap of the plants receiving nitrate nitrogen only was consistently higher than that of the plants receiving both nitrate and ammonium nitrogen. Also the plants supplied with both ammonium and nitrate nitrogen showed outstandingly less tendency to chlorosis than did the plants receiving nitrate nitrogen only.

These observations, as well as the numerous reports upon chlorosis in plants attributed to the accumulation of certain basic elements, such as calcium (1, 7, 8, 12), potassium (5, 22), and sodium (11, 17), suggested a quantitative investigation of the absorption and accumulation of basic elements by these corn plants, as influenced by the pH of the substrate and the form of nitrogen supplied. Accordingly, a study was made which involved the determination of the amounts of these base elements present in the expressed sap of the plants as well as the total quantities present in the tissues.

METHODS

The procedure used in growing these plants has been described in a previous publication (21). Briefly stated, the culture solutions were applied to the plants by the continuous flow method (18) of solution renewal, and in addition, every morning the sand of each culture was flushed with 2 liters of the nutrient solution adjusted to the required pH of the respective cultures.

In the analysis of the juice, an aliquot was evaporated to dryness on a steam bath, the organic matter oxidized with aqua regia, and the clear crystalline

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

residue made up to 100 ml. volume in 0.5 *N* HCl. Aliquots of this were taken for the mineral determinations.

The dried plant material was ground in a porcelain mortar and ball mill. Care was taken to prevent its contact with iron. Analyses were made on suitable aliquots of the pulverized tissue samples.

Potassium was determined according to the colorimetric procedure of Cameron and Failyer (2), which depends upon the development of the pink color of potassium iodoplatinate when an excess of potassium iodide is added to an aqueous solution of the potassium chloroplatinate precipitate.

Calcium and magnesium were determined strictly according to the procedure given by Frear and Kahlenberg (6).

The methods for scoring the plants for chlorosis, expressing the sap, and making the acidity determinations were discussed in the previous paper (21).

RESULTS

Plants grown with nitrate nitrogen only

Although the plants of the series receiving nitrogen in the form of nitrate only will be referred to as being grown at a given pH level, this stipulation is used only for convenience in referring to a culture supplied with nutrient solution at a given pH level. Although pH measurements were made on the "drip" solution from the drain holes of the percolator, no exact statement can be made with respect to the pH of the films on the absorbing surfaces of the roots in a given culture at any given time. In a later section of this paper a suggestion is made relative to the pH value of the solution films immediately in contact with these absorbing surfaces.

Table 1 shows the initial pH values of the solutions and the pH of the drip solution from the drain holes of the percolators just before and just after flushing. The marked tendency for plants to change the pH of the solutions in which they are growing has been observed many times. This tendency, as shown by table 1, was much in evidence in this investigation, yet, by virtue of the flushing procedure, the pH of the drip from the various cultures deviated less than 1 pH unit from that at which the solution was applied.

The total and "soluble" base content of these corn plants as influenced by the pH of the substrate is presented in table 2. The potassium content of these plants was found to be exceedingly high; that is, it varied from 4.8 to 7.8 per cent dry weight according to treatment. This value is several times that usually observed (10, 16) for the potassium content of field-grown corn plants; in fact, it was so abnormally high that the determinations were replicated several times in order to verify them. Potassium content was lowest in the plants grown at pH 3.0; markedly higher in the plants grown at pH 4.0; only slightly higher in plants grown at pH 5.0 than in those grown at pH 4.0; and then but little difference in potassium content was observed with further changes in pH of the growing medium above pH 5.0. The potassium content of the plants grown at pH 8.0, however, was noticeably lower than

that of plants grown at pH 5.0, 6.0, and 7.0. This was surprising, since, on theoretical grounds, it should be expected that cation absorption would increase with increase in the pH values of the substrate. Furthermore, the nutrient solutions were brought to the desired pH by the addition of potassium hydroxide, and therefore the potassium content of the pH 8.0 nutrient solution was actually four times that of the pH 4.0 solution, the diffusion gradient of this cation being thus proportionately increased. This indicates a probable optimum pH value of the substrate for the absorption of potassium under the

TABLE 1

Initial pH of culture solution containing nitrate nitrogen, and pH of the drip from drain hole of percolators at three different stages of plant development

pH of applied solution	pH of drip					
	August 1		August 8		August 15	
	Before flush	After flush	Before flush	After flush	Before flush	After flush
2.99	3.59	3.29	3.68	3.31	3.77	3.38
3.96	4.83	4.44	4.84	4.42	4.91	4.51
4.95	5.63	5.23	5.55	5.21	5.59	5.25
5.97	6.31	6.16	6.18	6.12	6.15	6.06
7.06	6.95	7.05	6.96	7.03	6.91	6.99
8.23	7.11	7.60	7.14	7.51	7.09	7.44

TABLE 2

Initial pH of culture solution and total and soluble base content, in per cent dry weight, of plants grown with nitrate as the sole source of nitrogen

pH of substrate	total K	soluble K	total Ca	soluble Ca	total Mg	soluble Mg
3.0	4.82	4.75	0.649	0.332	0.619	0.403
4.0	7.51	7.46	0.461	0.334	0.389	0.319
5.0	7.79	7.85	0.476	0.316	0.320	0.269
6.0	7.69	7.67	0.506	0.326	0.366	0.293
7.0	7.71	7.75	0.426	0.266	0.379	0.304
8.0	7.61	7.54	0.352	0.241	0.360	0.288

conditions here set up, an optimum which lies somewhere between pH 5.0 and 7.0, although the environment is made theoretically more conducive to the absorption of this cation by raising the pH of the substrate above the optimum here indicated.

In agreement with the observation of Morris and Sayre (13), the potassium content of these plants was found to be entirely soluble (filterable). This means that the entire potassium content was ionically active, and the importance of the effect which this may have had in the ionic equilibria of these plants cannot be stressed too greatly.

The calcium content of these corn plants varied from 0.35 to 0.65 per cent

dry weight according to treatment. This is somewhat lower than the usually observed (10, 16) calcium content of field-grown corn, and it contrasts markedly with the extreme "luxury consumption" of potassium. Undoubtedly, the high potassium absorption explains, in part, the comparatively low calcium absorption of these corn plants. Fonder (5) observed such an inverse relationship between calcium and potassium content in the alfalfa plant. Calcium accumulation was markedly the highest at pH 3.0—just the reverse of potassium accumulation—and was lowest at pH 8.0. Increase in pH of the nutrient solution was not accompanied, however, by a decrease in calcium accumulation over the entire range, since there was a secondary minimum in calcium absorption at pH 4.0 and a secondary maximum at pH 6.0, indicating that over a narrow portion of the pH range calcium intake tended to increase with increase in pH of the medium.

About 70 per cent of the calcium was "soluble," i.e., it was present in the filtered expressed juice, except in the plants grown at pH 3.0, where only about 50 per cent of the calcium was in the "soluble" form. This increased pro-

TABLE 3

Initial pH of culture solution and base content and titrable acidity, in milliequivalents per gram dry weight, of plants grown with nitrate as the sole source of nitrogen

pH of substrate	K	Ca	Mg	Total base	pH of expressed sap	Titrable acidity
3.0	1.23	0.324	0.508	2.06	5.41	0.552
4.0	1.92	0.230	0.320	2.47	5.62	0.410
5.0	2.00	0.238	0.263	2.50	5.68	0.424
6.0	1.97	0.253	0.300	2.52	5.72	0.440
7.0	1.98	0.213	0.311	2.50	5.74	0.478
8.0	1.95	0.176	0.300	2.43	5.69	0.366

portion of insoluble calcium in the pH 3.0 plants was accompanied by an increase in "insoluble" phosphorus, as indicated in the previous paper (21). It is quite conceivable that high accumulations of these two elements would favor mutual precipitation.

The magnesium content of the corn plants ranged between 0.320 and 0.619 per cent; which is well within the range of values usually reported for the magnesium content of corn grown under field conditions. Thus, the magnesium content of this corn was of the same order of magnitude as the calcium content; whereas the calcium content is usually considerably higher under field conditions (10, 16).

The magnesium content, like the calcium content, was highest in plants grown at pH 3.0; it was lowest in those grown at pH 5.0. Between pH 6.0 and pH 8.0 there was no significant variation in magnesium content. About 85 per cent of the magnesium was in the "soluble" form, indicating that the major portion of this element was in the ionically active condition.

Since it must be conceded that the chemical activity of these bases within the plant is dependent upon their presence in the ionically active form, it is quite apparent that a consideration of the base content in terms of equivalents per gram dry weight would present a better picture of the relative chemical activity of these elements than their consideration on the basis of percentage composition. Table 3 shows the relationship between the milliequivalents of the bases absorbed, the pH of the expressed juice, and the titrable acidity. It is quite evident that potassium makes up about 80 per cent of the total base equivalents per gram dry weight in plants of all treatments except those grown at pH 3.0, in which it made up about 60 per cent of the total base equivalents. Although table 2 showed that the percentage composition of calcium of these plants was only slightly higher than the magnesium content, it is quite manifest from table 3 that the equivalents of magnesium present per gram dry weight consistently exceed the equivalents of calcium present. This, of course, means that although magnesium may be found to be lower in percentage composition than calcium it is very apt to be more important than calcium in the electrovalent neutralizations involved in the maintainance of the observed pH of the plant cells.

The most interesting relationship brought out in table 3, is that between total milliequivalents of base present per gram dry weight and the pH of the expressed sap. Thus, there is consistently 2.5 m.e. of the total base elements per gram dry weight in the plants of all treatments except in those grown at pH 3.0, which contained 2.06 m.e., and in those grown at pH 8.0, which contained 2.43 m.e. The pH of the expressed sap of the plants of all treatments was approximately 5.7, except in those grown at pH 3.0 in which the pH of the expressed sap was 5.4. It appears highly improbable that such a close parallelism between total base equivalents and pH of the expressed sap could be pure chance.

The titrable acidity did not show so close a relationship to the total equivalents of base present as did active acidity. The plants grown at pH 3.0, however, contained the highest titrable acidity of the plants of all the series, and this is inversely correlated with the low base content of these plants.

Plants grown with both nitrate and ammonium as sources of nitrogen

Table 4 records the initial pH of the solution containing both nitrate and ammonium nitrogen, and the pH of the drain solutions just before and just after flushing of the cultures with 2 liters of the nutrient solution adjusted to the required pH of the respective cultures. It should be noted that it was the tendency of the plant in contact with the culture solutions to change the initial pH of the adjusted solutions toward a final value approximating pH 4.0, whereas the initial pH values of the solutions containing nitrate nitrogen only, tended to change toward neutrality under the influence of the growing plants. Trelease and Trelease (20) have pointed out the existence and physiological significance of these divergent effects produced by plants growing

in solutions containing nitrogen in the anion and in solutions containing nitrogen in the cation form.

Table 5 presents the values of the total and "soluble" base elements found in the plants of this series in terms of per cent of the dry weight. With the exception of the pH 3.0 cultures, the pH of the substrate appeared to have little influence on the potassium content of the plants. That is, the plants grown at the lowest pH level contained 3.4 per cent potassium, and the plants of the remaining five treatments contained amounts of potassium ranging between 5.7 and 6.4 per cent, with the maximum at pH 5.0. Since the pH

TABLE 4

Initial pH of culture solution containing both nitrate and ammonium nitrogen, and pH of drip from drain hole of percolators at three different stages of plant development

pH of applied solution	pH of drip					
	August 1		August 8		August 15	
	Before flush	After flush	Before flush	After flush	Before flush	After flush
2.97	3.36	3.16	3.25	3.11	3.05	3.02
4.04	4.42	4.18	4.27	4.08	3.95	3.99
4.98	5.65	5.25	5.32	5.11	4.57	4.78
6.02	6.32	6.12	6.11	6.03	5.49	5.81
7.05	6.99	7.01	6.69	6.82	6.43	6.72
8.29	7.22	7.45	7.08	7.41	6.81	7.46

TABLE 5

Initial pH of culture solution and total and soluble base content, in per cent dry weight, of plants grown with both nitrate and ammonium as sources of nitrogen

pH of substrate	total K	soluble K	total Ca	soluble Ca	total Mg	soluble Mg
3.0	3.38	3.29	0.392	0.217	0.572	0.373
4.0	5.89	5.84	0.249	0.208	0.327	0.269
5.0	6.41	6.43	0.324	0.215	0.298	0.253
6.0	6.32	6.30	0.318	0.202	0.314	0.261
7.0	6.09	6.12	0.294	0.183	0.326	0.270
8.0	5.71	5.66	0.238	0.141	0.283	0.237

of the nutrient solution was adjusted to the higher values by the addition of potassium hydroxide, making the potassium concentration in the pH 8.0 solutions nearly four times that in the pH 4.0 solutions, and since the H-ion concentration of the growth medium at pH 8.0 was, theoretically, more conducive to cation absorption than at the lower values, it is somewhat surprising to observe that in a series of cultures grown at pH levels ranging from 4.0 to 8.0 the lowest values for potassium accumulation should occur in the plants grown at the highest pH values. This represents another bit of evidence which suggests that the absorption and accumulation of nutrient ions in the

plant cannot be wholly due to concentration gradient, to membrane hydrolysis, or to both of these factors, but is, rather, the result of an intricate interaction of numerous complicated processes. It has been pointed out (3, 4, 14) that the absorption rate of ammonium nitrogen by plants is increased with increase in pH of substrate within certain limits, and it is possible that the rate of absorption of other cations may be similarly influenced. The limited experimental evidence along this line, however, may not warrant the acceptance, without further investigation, of the general principle here suggested that relatively high pH levels of the substrate are conducive to high rates of cation absorption and relatively low pH levels favor high rates of anion absorption.

The data of table 5 indicate that all the potassium present was in the "soluble" form; a fact which was pointed out also for the plants of the preceding series. The total potassium content of the plants grown with the solution containing both ammonium and nitrate nitrogen, however, was significantly lower than that of the plants of the preceding series which were grown with the solution containing nitrate nitrogen only. The presence of the ammonium ion apparently affects not only the rates of potassium absorption, but the absorption rates of calcium and magnesium, as well.

The calcium content of the plants of this series averages only about 65 per cent of the calcium content of the plants of the preceding series. Thus, it appears, as pointed out by Jacobson and Swanback (9), that the presence of ammonium retards calcium absorption. It appears to affect the absorption of this cation even more than it does that of potassium. The highest calcium content (0.39 per cent) was found in the plants grown with the pH 3.0 solution, and the lowest calcium content, in the plants supplied with the solution at pH 8.0, quite in contrast with the distribution of potassium. Approximately 65 per cent of the calcium in these plants was in the "soluble" form, indicating that a considerable proportion of this element had been precipitated within the plant tissues.

The distribution of magnesium in the plants of this series was similar to that of calcium. The content was highest (0.57 per cent) in the plants grown at pH 3.0 and lowest in those grown at pH 8.0. The magnesium content was appreciably lower in the plants of this series than in those of the preceding series, where nitrate was the sole source of nitrogen, although the difference was not nearly so marked as in the case of potassium and calcium. Approximately 80 per cent of the magnesium was present in the expressed sap.

In considering the milliequivalents of basic elements per gram dry weight, it is apparent from table 6 that potassium forms the major portion of the electropositive ions. The figures of especial interest, however, are those indicating the milliequivalents of total base per gram dry weight. The total base content of the plants grown at pH 3.0 was markedly the lowest of all values—1.53 m.e. per gram. There was little significant difference in the total base contents of plants grown at pH values ranging from 4.0 to 7.0, the observed quantities varying within limits of 1.92 and 2.05 m.e. per gram. The

total base content of the plants grown at pH 8.0 was somewhat lower—1.83 m.e. per gram. These values are all approximately 80 per cent of the corresponding values for the plants supplied with solutions containing nitrate nitrogen only.

In addition to the fact that the base contents of these plants were appreciably lower than those of plants grown at the corresponding pH levels of the preceding series, it will also be observed that the hydrogen-ion concentration of the expressed sap of the plants receiving ammonium and nitrate nitrogen was slightly but consistently higher than that of the plants receiving nitrate nitrogen only.

Consideration of the data of table 1 brings out the fact that the pH values of the culture solutions containing nitrate as the sole source of nitrogen for the plants, when adjusted to pH 6.0 were increased slightly above this value under the influence of the root absorption activities; when adjusted to pH 7.0 they were reduced slightly below this level by the action of the roots. This indicates an approximate dynamic pH equilibrium point somewhere between

TABLE 6

Initial pH of culture solution and base content and titrable acidity, in milliequivalents per gram dry weight, of plants grown with both nitrate and ammonium as sources of nitrogen

pH OF SUBSTRATE	K	Ca	Mg	TOTAL BASE	pH OF EXPRESSED SAP	TITRABLE ACIDITY
3.0	0.87	0.196	0.477	1.53	5.31	0.592
4.0	1.52	0.125	0.272	1.92	5.43	0.489
5.0	1.64	0.162	0.248	2.05	5.46	0.478
6.0	1.62	0.159	0.262	2.04	5.50	0.459
7.0	1.56	0.147	0.271	1.98	5.52	0.426
8.0	1.47	0.119	0.236	1.83	5.64	0.372

pH 6.0 and 7.0 toward which these plants under the experimental conditions here considered tended to reduce the pH values of solutions; the solutions which were adjusted to pH values below this dynamic equilibrium point increased in pH value and those adjusted to pH values above this point decreased under the influence of the plant roots. The solutions adjusted to pH 7.0 showed least change in contact with the plant roots, indicating that these were closest to the final equilibrium point under these experimental conditions.

Although a similar consideration of the data of table 4, as a whole, shows no well-defined final pH equilibrium point for the solutions containing both nitrate and ammonium nitrogen in contact with the plant roots, as might be expected, nevertheless, the data point to the fact that the final pH of the substrate resulting from contact with the plant roots lies considerably below the corresponding final pH of the solutions of the preceding series containing nitrate nitrogen only. Similar observations have been made by Tiedjens and Robbins (19).

Nightingale (15) has shown that the solution film immediately adjacent to

the absorbing surfaces of roots of young apple trees in sand culture may have a pH value very different from that of the body of the solution supplied to the roots. Thus the solution films on the absorbing surfaces of the apple roots were relatively acid (pH 4.2) when the plants were supplied with ammonium nitrogen in a solution having a pH value of 6.0. When the roots were supplied with nitrate nitrogen only in a solution at pH 4.5, the films on the surfaces of the actively absorbing roots were relatively less acid, showing a pH value of 5.6. It is thus evident that the final pH of a culture solution in contact with active plant roots, if not frequently or continuously renewed, will approach the pH of the solution films adjacent to the absorbing surfaces of the roots. In the final analysis the evidence appears to indicate that the pH of the solution films on the surfaces of the actively absorbing roots, as determined largely by the cation-anion nitrogen balance, has a much greater influence upon the nutrient ion intake and metabolic activity of the plant than has the pH of the body of the culture solution.

It is clear also that a more or less steep and variable hydrogen-ion concentration gradient between the root surface solution films and the body of the nutrient substrate is the usual condition under which plants develop, nor can it be doubted that such a condition must have a profound influence upon the absorption and accumulation of the nutrient ions by the plant. The full siginficance of this whole question of the effect of the external hydrogen-ion concentration upon absorption and accumulation of nutrient ions and upon the general metabolic activities of the plant will not be realized, however, without continued intensive investigation for some time to come.

SUMMARY

Corn plants were grown in two types of nutrient solutions, each adjusted to six different pH values ranging from pH 3.0 to pH 8.0. One type of culture solution contained nitrate nitrogen only, and the other type contained both nitrate and ammonium nitrogen in approximately equivalent proportions. Plants were analyzed for total and soluble potassium, calcium, and magnesium. Data for total and active acidity of the expressed sap of the plants are also given.

The total base content of all the plants was relatively high as compared with the usual base content value for soil-grown plants. There was a direct correlation between high potassium content and the pH of the expressed sap. The plants grown with the solution containing both nitrate and ammonium nitrogen in approximately equal proportions showed considerably lower total base content and lower pH of the expressed sap than did the plants grown with the solution containing nitrate nitrogen only.

The evidence indicates that the absorption of the ammonium ion lowers the rate of absorption of the other cations. The presence of ammonium in the nutrient solution had the most depressing effect on calcium absorption and the least effect on magnesium absorption.

The data indicate that high potassium absorption has a depressing effect on calcium and magnesium absorption.

The pH of the nutrient medium between the limits of pH 4.0 and pH 8.0 had little effect on base absorption and accumulation. The presence or absence of the ammonium ion had a much more marked effect upon absorption and accumulation of basic nutrients than had the variation in the pH levels of the substrate.

The evidence suggests that the pH values of the solution films adjacent to the absorbing plant roots in contact with solutions containing both nitrate and ammonium nitrogen were considerably lower than those of corresponding films on roots in contact with solutions containing nitrate nitrogen only, regardless of the pH levels of the body of the solutions. It is suggested that this phenomenon is partially responsible for the difference in base content shown by these two series of plants.

REFERENCES

(1) Burke, E. 1932 Chlorosis of trees. *Plant Physiol.* 7: 329–334.

(2) Cameron, F. K., and Failyer, G. H. 1903 The determination of small amounts of potassium in aqueous solutions. *Jour. Amer. Chem. Soc.* 25: 1063–1073.

(3) Clark, H. E., and Shive, J. W. 1934 The influence of the pH of a culture solution on the rates of absorption of ammonium and nitrate nitrogen by the tomato plant. *Soil Sci.* 37: 203–225.

(4) Davidson, O. W., and Shive, J. W. 1934 The influence of the hydrogen-ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand culture. *Soil Sci.* 37: 357–385.

(5) Fonder, J. F. 1929 Variations in potassium content of alfalfa due to stage of growth and soil type and the relationship of potassium and calcium in plants grown upon different soil types. *Jour. Amer. Soc. Agron.* 21: 732–750.

(6) Frear, D. E. H., and Kahlenberg, O. J. 1933 A study of the accuracy of the McCruddem method for calcium and magnesium in biological materials. *Jour. Biol. Chem.* 100: 85–95.

(7) Gile, P. L. 1911 Relation of calcareous soils to pineapple chlorosis. P. R. Agr. Exp. Sta. Bul. 11.

(8) Gile, P. L., and Carrero, J. C. 1916 Assimilation of iron by rice from certain nutrient solutions. *Jour. Agr. Res.* 7: 503–528.

(9) Jacobson, H. G. M., and Swanback, T. R. 1933 Relative influence of nitrate and ammoniacal nitrogen upon intake of calcium by tobacco plants. *Plant Physiol.* 8: 340–343.

(10) Latshaw, W. L., and Miller, E. C. 1924 Elemental composition of the corn plant. *Jour. Agr. Res.* 27: 845–861.

(11) Lipman, C. B. 1921 A contribution to our knowledge of soil relationships with citrus chlorosis. *Phytopath.* 11: 301–305.

(12) Mazé, P., Rout, and Lemoigne, M. 1912 Recherches sur la chlorose végétale provoquée par le carbonate de calcium. *Compt. Rend. Acad. Sci.* [*Paris*] 155: 435–437.

(13) Morris, V. H., and Sayre, J. D. 1935 Solubility of potassium in corn tissues. *Plant Physiol.* 10: 565–569.

(14) Naftel, J. A. 1931 The absorption of ammonium and nitrate nitrogen by various plants at different stages of growth. *Jour. Amer. Soc. Agron.* 23: 142–158.

(15) NIGHTINGALE, G. T. 1934 Ammonium and nitrate nutrition of dormant Delicious apple trees at 48° F. *Bot. Gaz.* 95: 437–452.

(16) PARKER, F. W., AND TRUOG, E. 1920 The relation between the calcium and the nitrogen content of plants and the function of calcium. *Soil Sci.* 10: 49–56.

(17) PETTINGER, N. A., HENDERSON, R. G., AND WINGARD, S. A. 1932 Some nutritional disorders in corn grown in sand cultures. *Phytopath.* 22: 33–51.

(18) SHIVE, J. W., AND STAHL, A. L. 1937 Constant rates of continuous solution renewal for plants in water cultures. *Bot. Gaz.* 84: 317–323.

(19) TIEDJENS, V. A., AND ROBBINS, W. R. 1931 The use of ammonia and nitrate nitrogen by certain crop plants. N. J. Agr. Exp. Sta. Bul. 526.

(20) TRELEASE, S. F., AND TRELEASE, H. M. 1933 Physiologically balanced culture solutions with stable H-ion concentration. *Science* 78: 438–439.

(21) WADLEIGH, C. H., ROBBINS, W. R., AND BECKENBACH, J. W. 1937 The relation between the chemical nature of the substrate and the degree of chlorosis in corn. *Soil Sci.* 43: 153–174.

(22) WALLACE, T. 1928 Investigations on chlorosis of fruit trees: II. The composition of leaves, bark and wood of current season's shoots in cases of lime induced chlorosis. *Jour. Pomol. and Hort. Sci.* 7: 172–183.

IS SUNLIGHT A FACTOR IN NITROGEN TRANSFORMATION IN SOIL?

B. N. SINGH AND K. M. NAIR

Institute of Agricultural Research, Benares Hindu University, India

Received for publication September 16, 1938

Considerable attention was recently drawn to the fact that agencies other than the soil microflora are concerned in the processes of ammonification and nitrification in soils. The rôle of the chemical reactions induced by light has been claimed by Dhar (5) to be of great significance in tropical soils in bringing about the oxidation of organic nitrogenous materials to ammonia, nitrite, and nitrate successively. In this connection it is expedient to recall the investigations, recently discussed by Waksman and Madhok (14), which demonstrate that photoöxidation is limited to the oxidation of ammonia to nitrite, except when the pH of the medium is far below that normally occurring in soils. Moreover, attempts made in India (4) and in America (11, 14) failed to show that photonitrification was of any significance in the accumulation of nitrate in soils. The lack of correlation between bacterial numbers and nitrification observed in the Malay Archipelago and the increased rates of nitrate formation in summer in India are cited by Dhar (6) in support of his view. Of course it might also be concluded from these data that photonitrification is responsible for nitrate accumulation in soils, provided meteorological and edaphic factors, other than light, do not interfere. This new phenomenon identified in soils has caused much conflict, and, in view of its importance in the availability of soil nitrogen, further work seems essential. Accordingly an attempt was made at this research station to answer the following questions:

1. Does photonitrification occur? If so, what is its significance in nitrate accumulation in soils?
2. Does light influence all the reactions involved in the processes of ammonification and nitrification, namely, the oxidation of organic nitrogenous substances to ammonia, of ammonia to nitrite, and of nitrite to nitrate?

EXPERIMENTAL METHOD

Transformations of the nitrogen radicals of various materials were tested separately in sterilized distilled water, in sterilized soil, and in reinoculated sterilized soil. The substances chosen were dried blood, Merck's ammonium sulfate, sodium nitrite, and sodium nitrate, which contain radicals of nitrogen analogous in composition to those of the soil's nitrogen reserve and its subsequent oxidation products. Each treatment was in triplicate pairs. One set

of the members of each pair was exposed to sunlight, and the other was kept in a lightproof chamber. This method of protecting the treatments from light was adopted in order to avoid large thermal variations generally associated with the blackening of the surfaces of the containers (10). The rate of addition of the substances was 50 mgm. of nitrogen for 100 gm. of substrate. The period of experimentation was 4 weeks in each case.

Where sterilized distilled water was the medium, it was kept in 750-cc. Erlenmeyer flasks provided with two-holed stoppers fitted with tubes for aeration, which was supplied on alternate days for 5 minutes. The flasks and contents were sterilized in an autoclave for 1 hour at 20 pounds pressure per square inch. Air was freed from ammonia, from oxides of nitrogen, and from microörganisms by washing through sulfuric acid, sodium hydroxide, and mercuric chloride solutions. The open ends of the tubes were plugged with sterilized cotton wool.

The soil used was a heavy loam and was standardized in the usual manner before experimentation. Containers used were 500-cc. Pyrex glass beakers with petri-dish covers. In one set of experiments the soil was sterilized in an electric oven for 2 hours at 150 $\pm$ 5°C. to eliminate the influence of microorganisms. In another set it was sterilized and reinoculated with a uniform suspension of well-ground fresh garden soil in distilled water. Triplicate controls, without additional nitrogen, were set up for each treatment. Both in the series kept in the dark and in the series exposed to sunlight, equal temperature was maintained by baths containing water at 28° to 33°C. which came from a common reservoir kept in the shade. Moisture content was made up to 33 per cent of the water-holding capacity by adding sterilized distilled water, and the amount of water lost by evaporation was added every third day. The reaction of the soil was brought up to pH 8 by the addition of lime; pH determinations were made by means of a Hartman and Braun potentiometer. Uniformity of aeration in the different samples was maintained by tamping the soil to the same level in the beakers.

To ensure further accuracy in the net results, the nitrogenous impurities in the various reagents were estimated, and due deductions were made.

Ammoniacal nitrogen was estimated by the method of Harper (12). Nesslerization was resorted to whenever the quantity of ammonia distilling out was less than 2 mgm.

Nitrous nitrogen was determined by using the Griess Ilosvay reagent (15). Color comparisons were made by means of a Dubosq colorimeter.

Nitric nitrogen was estimated by the phenoldisulfonic acid method (2, p. 12). To eliminate errors due to the presence of nitrous nitrogen, an adaptation of the A. P. H. A. method (1, p. 20) was resorted to.

Another set of experiments with sterilized soil and ammonium sulfate was instituted on essentially the same lines as the aforementioned. Periods of exposure to sunlight were varied, and corresponding amounts of solar radiation incident to the treatments were measured by means of a Callendar's sunshine

recorder set up at the meteorological observatory of this research station. The treated materials were kept in the vicinity of the apparatus.

EXPERIMENTAL FINDINGS

No ammonification occurred in the sterilized materials whether exposed to sunlight or kept in the dark (table 1). Dried blood in sterilized water or sterilized soil did not show the least trace of ammonia. Reduction of nitrite

TABLE 1

Net amounts of nitrogen transformation in treatments in dark and in sunlight

Milligrams per 100 gm. of substrate; duration 4 weeks

SUBSTRATE	TREATMENT	AMMONIFICATION* NH_4–N		NITRITE FORMATION* NO_2–N		NITRATE FORMATION* NO_3–N	
		Dark	Sunlight	Dark	Sunlight	Dark	Sunlight
Sterilized distilled water	Dried blood	0	0	0	0	0	0
	$(NH_4)_2SO_4$	0	0	0	3.04	0	0
	$NaNO_2$	0	0	0	0	0.15	0
	$NaNO_3$	0	0	0	1.63	0	0
Sterilized soil	Initial nitrogen	0	0	0	0	0	0
	Dried blood	0	0	0	0	0	0
	$(NH_4)_2SO_4$	0	0	0	6.74	0	0
	$NaNO_2$	0	0	0	0	0	0
	$NaNO_3$	0	0	0	0	0	0
Reinoculated sterilized soil	Initial nitrogen	17.86	17.21	8.24	14.32	8.04	8.32
	Dried blood	35.79	36.12	18.94	25.52	18.16	17.94
	$(NH_4)_2SO_4$	16.96	17.02	18.76	24.49	18.34	18.59
	$NaNO_2$	21.23	21.74	9.14	16.76	8.91	8.73
	$NaNO_3$	29.35	29.62	11.31	18.44	9.15	9.59

* Ammonification = final (NH_4–N + NO_2–N + NO_3–N)—initial (NH_4–N + NO_2–N + NO_3–N).

Nitrite formation = final (NO_2–N + NO_3–N)—initial (NO_2–N + NO_3–N).

Nitrate formation = final (NO_3–N)—initial (NO_3–N).

The untreated soil had no initial NH_4–N and NO_2–N, but it had 5.16 mgm. NO_3–N and 65.02 mgm. organic nitrogen per 100 gm.

or nitrate to form ammonia did not occur in the respective solutions. After the soils were sterilized, the initial nitrogen also remained stable during experimentation. The addition of various salts did not produce any change in initial nitrogen of the soil. Thus the complete immobility of the process of ammonification in sterilized media, in spite of irradiation with sunlight, definitely excludes the possibility of photolysis of organic nitrogenous materials and indicates that some other agency is concerned.

By reinoculation, organisms were introduced in equal numbers to the vari-

ously treated sterilized soils, and in such cases the results testify to the progress of ammonification. The soils receiving no treatments showed signs of the decomposition of their initial nitrogen. Virtually the same quantities of ammonia were produced in corresponding treatments in the dark and exposed to the sun. The addition of dried blood to the reinoculated sterilized soil increased the rate of ammonification, but the process showed lack of response to sunlight. Different treatments affected the rate of ammonification of the initial nitrogen reserve of the soil as follows: sodium nitrate produced the greatest effect, followed in order by sodium nitrite and ammonium sulfate. Variations in the production of ammonia in the covered and exposed treatments were so slight that little influence could be attributed to sunlight in the process of ammonification.

In a sterilized suspension of dried blood, no nitrite was formed. Nitrate formation took place in exposed solutions of ammonium sulfate and of sodium nitrate, but at a greater rate in the former. This demonstrates the effect of sunlight in oxidizing ammonia to nitrite and in reducing nitrate to nitrite. The reduction processes, however, were less rapid than the photoöxidation process. Nitrite solution remained stable in light. In sterilized soil medium there was rapid oxidation of ammonium sulfate in the exposed treatments, and the rate was greater than that in solutions. The absence of nitrous nitrogen formation in sterilized treatments in the dark confirms the importance of light and helps to ascertain its influence in a quantitative manner. In solutions, nitrate underwent reduction to nitrite to a slight extent, but in sterilized soil this photochemical reduction either was completely inhibited or the traces of nitrous nitrogen formed were locked away in the soil.

Nitrate formation, whether from the initial nitrogen of the soil or from dried blood, was manifest both in the covered and in the exposed treatments which were inoculated. The occurrence of this process in the dark proves that microörganisms are independently capable of transforming ammoniacal nitrogen to nitrous nitrogen. The increased rates of oxidation in the treatments exposed to sunlight again showed the significant influence of solar radiation in the formation of nitrous nitrogen in the soil. Variations denoting the specificity of the treatments were also evident from the high rates of nitrous nitrogen formation from dried blood and ammonium sulfate, the low production from the initial nitrogen of the soil, and the medium results in the treatments receiving sodium nitrite and nitrate. Though the treatments showed specific rates, the excesses in favor of sunlight remained without significant variations among themselves. From this it may be concluded that the efficiency of sunlight in the photoöxidation of ammonia in soils has quantitative limits, irrespective of the variation in treatments.

Except for a solution of sodium nitrite in the dark, nitrous nitrogen did not oxidize to nitric nitrogen in sterilized treatments, whether in distilled water or in soil, in sunlight or in the dark. The oxidation of sodium nitrite

in the absence of light could be considered only as a physicochemical phenomenon. Such oxidation was very slow, and in sterilized soil it was inhibited or, if it occurred, was difficult to estimate. When the soil treatments were reinoculated, measurable amounts of nitrate were formed. Comparison of the treatments in light with those in dark shows no correlation between nitrate formation and exposure to sunlight. Thus the oxidation of nitrous nitrogen to nitric nitrogen is entirely a biological reaction which does not seem to respond to sunlight. The variations in the results among the different treatments indicate the specificity of the materials employed.

With increasing incidence of solar radiation, increased quantities of nitrous nitrogen were formed (table 2) from ammonium sulfate in sterilized soil. The oxidation of ammonia to nitrous nitrogen is directly proportional to the amounts of solar radiation received by the treatments. Thus the photochemical nature appears to be very patent in this phase of the chain of reactions involved in the mineralization of soil nitrogen.

TABLE 2

Nitrite formation from ammonium sulfate in sterilized soil in relation to the incidence of solar radiation

SOLAR RADIATION	NITROUS NITROGEN
cal./sq.cm.	*mgm./100 gm. soil*
6,437	3.45
12,870	6.74
19,305	10.16
25,742	14.01

DISCUSSION

The investigations reported define clearly the influence of photochemical and biological activities on the reactions involved in the processes of ammonification and nitrification in soil.

In experiments with sterilized distilled water as the substrate, the observations recorded for photochemical transformations were not affected by the various complex factors generally associated with soils. The results in such experiments manifest the influence of light in the oxidation of ammonia to nitrite and in the reduction of nitrate to nitrite. Rao (13), however, has shown that organic nitrogen can be photochemically oxidized to ammonia, nitrite, and nitrate successively. On the other hand, Berthelot and Gaudechon (3) observed the photoöxidation of ammonia to nitrite and no further. They also observed an identical reduction of nitrate on exposure to light as reported in this investigation. The instability of nitrate together with the absence of its synthesis, in light, definitely points to the fact that photochemical reactions are of little significance in nitrate formation.

In experiments with sterilized soil as the substrate, the photoöxidation of ammonia to nitrite is also reported. The stimulating influence of light on the oxidation in such experiments leads to the obvious conclusion that the soil acts as a photocatalyst in the reaction. That the conversion to nitrite is not purely a photochemical process is borne out by the fact that nitrite is formed in the covered reinoculated soil treatments. Moreover, the excess quantities of nitrite formed in the exposed soil cultures which were inoculated suggest the influence of light in addition to the biological oxidation of ammonia to nitrite.

The sphere of photochemical processes in transformations of nitrogen in soil having thus been established, a comparison may be made with bacterial activity. The oxidation of organic nitrogenous material to ammonia and of nitrite to nitrate was found to be stationary in sterilized media. When the soils were reinoculated, these processes became dynamic, proving thereby the indispensability of microörganisms in nitrogen changes. Hence the mechanism of nitrogen transformation in soil seems to be a combination of photochemical and biological phenomena. The biological processes disintegrate the organic nitrogen to ammonia, the combined influence of light and bacteria is manifest in the oxidation of ammonia to nitrite, and further oxidation of nitrite to nitrate is entirely biological.

That additional quantities of nitrite formed as a result of photochemical oxidation in soil cultures do not in any way contribute to the greater accumulation of nitrates is evident from the fact that both exposed and covered treatments show the same rate of nitrate formation. Thus the significant transformations effected by sunlight in a limited part of the chain of reactions involved are of no significance in the final accumulation of nitrate in soil.

Contrary to the observations of Dhar et al. (5, 6, 7, 8, 9, 10), the biological reactions seem to have great significance in the processes of ammonification and nitrification in soil, though sunlight stimulates the oxidation of ammonia to nitrite to a considerable extent in soil.

SUMMARY

An attempt has been made to compare the extent to which photochemical and biochemical phenomena are associated with the processes of ammonification and nitrification in soil.

Various nitrogenous substances were tested in exposed and covered treatments in sterilized and in unsterilized substrates.

The experimental technic concerned in the protection of treatments from light was so devised as to avoid large thermal variations generally associated with the blackening of the surfaces of containers.

The accumulation of nitrite was due to both photochemical and biological reactions.

The oxidation of nitrite to nitrate was exclusively due to biological agency.

The conclusion reached is that biological reactions are largely responsible for the processes of ammonification and nitrification in soil, though nitrite is formed also as a result of photochemical action.

REFERENCES

(1) American Public Health Association 1925 Standard Methods for Examination of Water and Sewage, ed. 6. New York.

(2) Association of Official Agricultural Chemists 1930 Methods of Analysis, ed. 3. Washington D. C.

(3) Berthelot, D., and Gaudechon, H. 1911 La nitrification par les rayons ultra-violets. *Compt. Rend. Acad. Sci.* [Paris] 152: 522–524.

(4) Desai, S. V., and Fazal-ud-Din 1934 The nature of nitrification in soil. *Indian Jour. Agr. Sci.* 6: 777–783.

(5) Dhar, N. R. 1935 Influence of Light on Some Biochemical Processes. Soc. Biol. Chem. (Indian Inst. Sci.), Bangalore.

(6) Dhar, N. R. 1935 Influence of light on the nitrogen cycle in the soil. *Sci. and Culture* (I) 4: 243–246.

(7) Dhar, N. R., Bhattacharya, A. K., and Biswas, N. N. 1933 Photonitrification in soil. *Soil. Sci.* 35: 281.

(8) Dhar, N. R., Bhattacharya, A. K., and Biswas, N. N. 1933 Influence of light on ammonification and nitrification in soil. *Jour. Indian. Chem. Soc.* 10: 699.

(9) Dhar, N. R., and Rao, G. G. 1933 Nitrification in soil and air, a photochemical reaction. *Jour. Indian Chem. Soc.* Ray Comm. Vol., p. 81.

(10) Dhar, N. R., and Tandon, S. P. 1934 Influence of temperature on bacterial nitrification in tropical soils. *Soil Sci.* 38: 183–189.

(11) Fraps, C. S., and Sterges, A. J. 1935 Effect of sunlight on the nitrification of ammonium salts in soils. *Soil Sci.* 39: 85–94.

(12) Harper, H. J. 1937 Method of estimation of ammonia in soils. *Soil Sci.* 44: 361–375.

(13) Rao, G. G. 1934 Newer aspects of nitrification: I. *Soil Sci.* 38: 143–159.

(14) Waksman, S. A., and Madhok, M. R. 1937 Influence of light and heat on formation of nitrate in soil. *Soil Sci.* 44: 361–375.

(15) Wright, H. C. 1934 Soil Analysis. Thomas Murby and Co., London.

HOST PLANT SPECIFICITY AMONG THE MEDICAGO[1] IN ASSOCIATION WITH ROOT-NODULE BACTERIA

J. C. BURTON AND P. W. WILSON

University of Wisconsin

Received for publication October 10, 1938

In a previous report[2] experiments were described which demonstrated that nitrogen fixation by *Melilotus* sp. is determined not only by the strain of *Rhizobium meliloti* which invades the plant but likewise by the species of the host. Because of the significance of the occurrence of this specificity of the host plant in the symbiotic nitrogen fixation system from both a theoretical and a practical point of view, the investigations have been extended to the *Medicago*.

METHODS

Different species of *Medicago* which had been inoculated with various strains of *Rh. meliloti* were grown in half-gallon glazed jars on a pit-sand substrate to which all necessary plant nutrients except combined nitrogen were added. After 2 to 3 months in the greenhouse the plants were harvested and analyzed for *dry weight* and *total nitrogen*. In some experiments a modification of this technic was introduced. Two-gallon jars equipped with watering tubes were seeded with four different species (or varieties), one to each quadrant of the jar. All the plants in a single jar were inoculated with the same strain of the organism. This technic insured identical conditions of growth for each of the four plant species tested. Details of the methods used for growth and for analysis of the plants are given in the previous communication.

The following species of *Medicago* were used in the experiments:[3]

Medicago arabica Huds.	Annual spotted medick
Medicago hispida Gaertn.	Annual bur clover
Medicago lupulina L.	Annual black medick
Medicago minima L.	Annual fine stemmed black medick
Medicago sativa L.	Perennial alfalfa (lucerne)
Grimm	
Ladak	
Hairy Peruvian	

[1] Herman Frasch Foundation in Agricultural Chemistry, Paper no. 182. Contribution from the departments of agricultural bacteriology and biochemistry, University of Wisconsin. Technical assistance on this problem was supplied in part by workers employed in the University's Works Progress Administration Natural Science Project.

[2] Wilson, P. W., Burton, J. C., and Bond, V. S. 1937 Effect of species of host plant on nitrogen fixation in *Melilotus*. *Jour. Agr. Res.* 55: 619–629.

[3] The authors express their appreciation to the Bureau of Plant Industry, Division of Forage Crops and Diseases, U. S. Department of Agriculture, for its aid in securing the seeds of these *Medicago* species and varieties.

A summary of the chief characteristics of these species is given in Gray's *Manual of Botany*. The strains of the organism which were used for inoculation of the plants were those employed in the experiments with *Melilotus*; a description of these strains was supplied in the previous publication.

EXPERIMENTAL

In a preliminary experiment three varieties of *Medicago sativa* (Grimm, Ladak, and Hairy Peruvian) were inoculated with five strains of *Rh. meliloti*. The planting was made on January 15, and the harvest on March 7. During most of the growth period the weather was cloudy, and illumination was very low; consequently, growth was poor. At harvest, duplicate cultures, which were grown in the half-gallon jars, were combined for analysis. Consideration

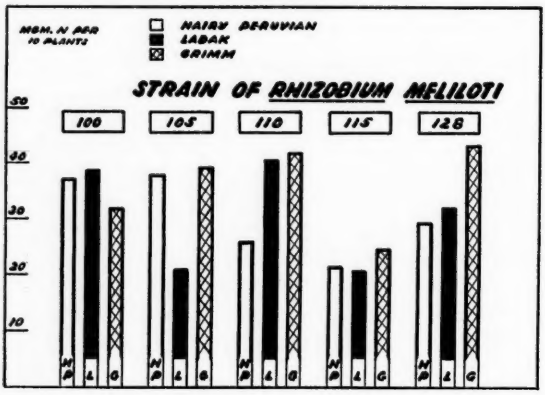


FIG. 1. EFFECT OF STRAIN OF RH. MELILOTI ON NITROGEN FIXATION BY VARIETIES OF MEDICAGO SATIVUM

(Preliminary experiment, January 15 to March 7)

of the nitrogen fixation data, which are presented in figure 1, indicates that some degree of host plant specificity exists among these three varieties of *M. sativa* with strains 105, 110, and 128, but the differences are not large. Further trials were accordingly made under more suitable environmental conditions; in these, the replicate samples were analyzed separately in order that the data might be subjected to statistical analysis. The details of these experiments are as follows:

Experiment 1. Five species of *Medicago* including three varieties of *M. sativa* and the five strains of bacteria employed in the preliminary experiment were used. The planting was made in early spring, and growth conditions during the course of the experiment (April 16–June 3) were quite satisfactory. The development of the plants is shown in plate 1; the chief analytical data are given in tables 1 and 3.

Experiment 2. This experiment was identical with experiment 1 except that *M. minima*

TABLE 1

Effect of strain of rhizobia on nitrogen fixation by Medicago sativa, *experiments 1 and 2, April 16 to July 11**

VARIETY OF MEDICAGO SATIVA	STRAINS OF RH. MELILOTI				
	100	105	110	115	128
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
			Experiment 1		
Hairy Peruvian	35.4	25.2	37.2	17.0	24.3
	42.2	29.7	30.2	23.8	21.2
	38.4	14.9	35.4	20.0	31.9
	38.7	*23.3*	*34.3*	*20.3*	*25.8*
Ladak	32.5	27.4	39.3	37.3	32.7
	47.0	12.6	45.0	42.5	18.2
	46.6	15.3	34.8	35.6	35.4
	42.0	*18.4*	*39.7*	*38.5*	*28.8*
Grimm	43.8	20.0	55.5	21.2	49.2
	46.2	10.2	36.4	25.2	23.0
	45.0	17.8	42.6	30.0	27.3
	45.0	*16.0*	*44.8*	*25.5*	*33.2*
Total.....	376.9	173.3	356.5	252.8	262.6
			Experiment 2		
Hairy Peruvian	33.0	26.9	42.7	14.1	10.6
	50.1	17.4	40.0	10.1	11.4
	41.6	*22.1*	*41.3*	*12.1*	*11.0*
Ladak	42.0	18.2	12.3	46.0	25.9
	46.5	8.7	38.3	44.8	34.8
	44.3	*13.5*	*25.3*	*45.4*	*30.4*
Grimm	42.0	18.9	21.4	44.2	25.3
	45.6	10.1	45.0	30.7	16.8
	43.8	*14.5*	*33.2*	*37.5*	*21.1*
Total.....	259.2	100.2	199.7	190.5	124.8

* Data in tables 1, 2, and 3 in mgm. N per 10 plants. Italicized values are means of replicates.

Differences necessary for significance: between means—experiment 1, 15.6 mgm.; experiment 2, 18.2 mgm.; between totals of varieties—no significant differences; between totals of strains—experiment 1, 81.3 mgm.; experiment 2, 43.4 mgm.

The total values given in tables 1, 2, and 3 for variety (or species) of *Medicago* represent the total for all strains of the bacteria; similarly, each total given for the strains represents that for all varieties (or species) of the host plant. If two totals differ by the value indicated as necessary for significance, the odds are at least 19:1 that the observed difference did not arise by chance (experimental error). Totals not supplied indicate that the statistical analysis showed that the differences were not significant.

was not included, and the plants were grown in the 2-gallon jars, four species or varieties being sown in each jar. Tables 1 and 3 summarize the results.

Experiment 3. The three varieties of *M. sativa* which were taken as the host plants were grown together in the 2-gallon jars. Seven strains of the organism were used, including four which had not been previously tested. The experiment was started July 1. During the first 2 months the weather was very hot and, in general, unfavorable for the growth of greenhouse plants. The essential data are given in table 2.

The data in the tables show definitely that host plant specificity occurs among the species of *Medicago* and possibly also among the varieties of *M. sativa*. Variation among some of the replicate samples was too great, however,

TABLE 2

Effect of strain of rhizobia on nitrogen fixation by Medicago sativa, *experiment 3, July 1 to October 5**

VARIETY OF MEDICAGO SATIVA	STRAIN OF RH. MELILOTI							
	100	101	105	107	111	115	129	Total
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Hairy Peruvian	40.0	33.9	25.0	30.0	26.5	24.8	26.8	334.8
		34.6	32.6	33.1	13.3	13.3	40.8	
		34.3	*28.8*	*31.6*	*19.9*	*19.1*	*33.8*	
Ladak	28.2	6.8	23.2	30.0	39.5	15.0	24.8	254.6
		7.3	18.7	16.4	36.0	16.3	20.2	
		7.1	*21.0*	*23.2*	*37.8*	*15.7*	*22.5*	
Grimm	38.8	27.2	37.7	31.0	21.9	19.5	27.2	329.4
		33.8	26.2	28.9	21.7	19.3	35.0	
		30.5	*32.0*	*30.0*	*21.8*	*19.4*	*31.1*	
Total.....		143.6	163.4	169.4	158.9	108.2	174.9	

* Differences necessary for significance: between means, 11.35 mgm.; between totals of varieties, 55.6 mgm.; between totals of strains, 39.3 mgm.

to establish conclusively, without statistical analysis, the significance of the differences noted among the varieties. Accordingly, the complete data were subjected to an analysis of variance.[4] A summary of these analyses are given in table 4; before discussion of the individual experiments, the following points concerned with the interpretation of the statistical tests should be noted:

If the variance due to *species* (or *variety*) is significant, it indicates that at least one species (or variety) fixes more nitrogen with *all* the strains used than do the others. With a given strain of the organism the superior species may not fix so much nitrogen as does another species, but the *total fixed with all the strains* is significantly higher than is the total of any

[4] Fisher, R. A. 1930 Statistical Methods for Research Workers, ed. 3. Edinburgh and London.

other species. Consideration will show that this source of variation may not be too important, since a given species may fix large quantities of nitrogen when inoculated with four strains of the organism but fix very little with a fifth strain. A second species might fix only medium quantities of nitrogen with all five strains, and yet the total fixation, all the strains being considered, might be significantly higher than is that of the first species. In such a case the conclusion that the second species is superior from the point of view of nitrogen fixation is obviously unwarranted.

If the variance due to *strain* is significant, it means that the *total nitrogen* fixed by at least one strain of the organism on the *entire lot* of host plants is greater than is that fixed by any other strain. Knowledge of this source of variation might be of importance in preparation of a culture for distribution in an area where different species of the host plant were being used.

The variance which is of particular importance for the host specificity problem is that due to interaction of *strain* and *species* (or *variety*). If this source of variation is significant, it indicates that fixation of nitrogen by a given strain of the organism depends not only on the strain itself but also on the species of the plant which serves as the host. If host plant specificity is thus established, examination of the original data from the point of view of differences between the means will determine which strains and species are involved.

DISCUSSION

Varieties of M. sativa

In experiment 1 the only significant source of variation was that due to *strain*. Strains 100 and 110 fixed significantly more nitrogen than did any of the others, and strain 105 fixed least. These results agree with those previously obtained with these strains when they were used to inoculate different species of *Melilotus*; with host plants of this latter genus strain 100 was the most efficient, and strain 105 the least efficient. Examination of the data (table 1) suggests the existence of host plant specificity, but the analysis of variance failed to confirm this, mainly because the wide variation among some of the replicates caused an appreciable *variance due to error*. As a result, the difference between any two means would have to be very large in order to reach the level of significance.

In experiment 2 the differences between the means were considerably higher than those in experiment 1, and host plant specificity was readily established. Strain 100 was very efficient, and strain 110 was fairly efficient on all varieties, but both strains 115 and 128 fixed less nitrogen in association with *Hairy Peruvian* than with the other two varieties tested. Strain 105 appeared to be poor with all hosts and to show some evidence of host plant specificity, but fixation was too low and erratic on all the varieties to establish this, in view of the comparatively large experimental error. The variance due to *strain* was again significant, as in experiment 1. Strain 100 was once more superior on all varieties.

In experiment 3 (table 2) again host plant specificity was evident among the varieties of *M. sativa*, particularly with strains 101 and 111. Results of nitrogen fixation experiments with strain 101, which is usually regarded as a "poor"

TABLE 3

Effect of strain of rhizobia on nitrogen fixation by species of Medicago, *experiments 1 and 2, April 16 to July 11**

SPECIES OF MEDICAGO	STRAIN ON RHIZOBIUM MELILOTI					
	100	105	110	115	128	Total
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
			Experiment 1			
M. arabica	1.2	17.2	1.1	1.3	1.2	
M. minima	53.6 53.4 *53.5*	35.9 31.9 *33.9*	76.2 — *76.2*		40.8 41.4 *41.1*	
M. hispida	1.2 1.1 *1.2*	33.9 35.8 *34.9*	1.2 1.3 *1.3*	1.8 1.2 *1.5*	1.6 1.5 *1.6*	80.6
M. lupulina	32.7 41.1 *36.9*	72.7 79.2 *76.0*	46.4 57.2 *51.8*	69.5 36.1 *52.8*	57.4 63.3 *60.4*	555.6
M. sativa (Grimm)	*45.0*	*16.0*	*44.8*	*25.5*	*33.2*	328.6
Total	166.1	253.6	195.7	159.6	190.2	
			Experiment 2			
M. arabica	1.2 1.4 *1.3*	51.6 42.6 *47.1*	2.6 3.4 *3.0*	3.8 2.3 *3.1*	4.5 2.5 *3.5*	116.0
M. hispida	1.7 2.6 *2.2*	56.6 48.6 *52.6*	5.1 2.4 *3.8*	3.2 2.2 *2.7*	1.4 1.7 *1.6*	124.9
M. lupulina	27.0 34.9 *31.0*	63.0 75.6 *69.3*	76.7 78.0 *77.4*	40.0 37.6 *38.8*	97.0 30.5 *63.8*	560.3
M. sativa (Grimm)	*43.8*	*14.5*	*33.2*	*37.5*	*21.1*	300.0
Total	156.4	366.5	234.6	164.0	179.7	

* Differences necessary for significance: between means—experiment 1, 15.15 mgm.; experiment 2, 24.95 mgm.; between totals of varieties—experiment 1, 67.7 mgm.; experiment 2, 111.5 mgm.; between totals of strains—experiment 1, 52.3 mgm.; experiment 2, 99.8. Statistical analysis in experiment 1 based on data for *M. hispida*, *M. lupulina*, and *M. sativa* (Grimm); in experiment 2, *M. arabica* was also included. Detailed data for *M. sativa* (Grimm) given in table 1.

strain, are often very erratic. With these particular strains of *Rh. meliloti* there was a significant difference due to *variety*, *Ladak* fixing less total nitrogen

with all strains than did the other two. Among the strains, 115 fixed less total nitrogen on all varieties than did any of the others.

Altogether, these results agree satisfactorily with those previously obtained by using the same strains of the organism with *Melilotus* species. The exis-

TABLE 4

Summary of analyses of variance of individual experiments

VARIANCE DUE TO	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	*F*	5 PER CENT POINT	1 PER CENT POINT	SIGNIFICANCE*
I. Varieties of *Medicago sativa*							
Experiment 1							
Varieties	2	222.7	111.4	1.26	3.32	—	—
Strains	4	3,063.5	765.9	8.70	2.69	4.02	++
V × S	8	509.3	63.7	0.72	3.00	—	—
Error	30	2,641.6	88.1	—	—	—	
Experiment 2							
Varieties	2	194.5	97.3	1.34	3.68	6.36	—
Strains	4	2,675.8	668.9	9.35	3.06	4.89	++
V × S	8	1,722.8	215.3	2.90	2.64	4.00	+
Error	15	1,090.4	72.7	—	—	—	
Experiment 3							
Varieties	2	616.1	308.1	10.51	3.55	6.01	++
Strains	5	775.6	155.1	5.28	2.77	4.25	++
V × S	10	1,001.8	100.2	3.41	2.42	3.54	+±
Error	18	527.4	29.3	—	—	—	
II. Species of *Medicago*							
Experiment 1							
Species	2	11,286.6	5,643.3	112.3	3.68	6.36	++
Strains	4	965.9	241.5	4.80	3.06	4.89	+±
S × S	8	3,525.0	440.6	8.76	2.64	4.00	++
Error	15	754.2	50.3	—	—	—	
Experiment 2							
Species	3	12,983.2	4,327.7	30.6	3.10	4.94	++
Strains	4	3,810.0	952.5	6.68	2.87	4.43	++
S × S	12	7,707.7	642.3	4.50	2.28	3.23	++
Error	20	2,851.4	142.6	—	—	—	

* If the value of *F* for a given source of variance exceeds the value given for the 5 per cent point, the odds are at least 19:1 that the variation is *significant* (denoted by +). This means that the variance arose from treatment, *e.g.*, use of different strains of organism for inoculation, and not from chance (experimental error). Likewise if the value of *F* exceeds the 1 per cent point, the odds are at least 99:1 that the variation did not arise from chance (highly significant, denoted by ++). If observed variation could have arisen from chance, — is used.

tence of host plant specificity was not so clear-cut, however, with the varieties of *M. sativa* as with the species of sweet clover. With the varieties of alfalfa tested, the efficiency of certain of the individual strains varied among the experiments, an effect previously discussed in connection with the experiments

on *Melilotus*. The occurrence of this type of variation tends to obscure host plant specificity.

Species of Medicago

A striking example of host plant specificity is furnished by the action of the different strains of *Rh. meliloti* on the species of *Medicago* tested (table 3 and plate 1). The analyses of variance summarized in table 4 show that in both experiments 1 and 2, the variances due to *variety* and *strain* as well as to the *interaction* between these two sources of variation were highly significant.

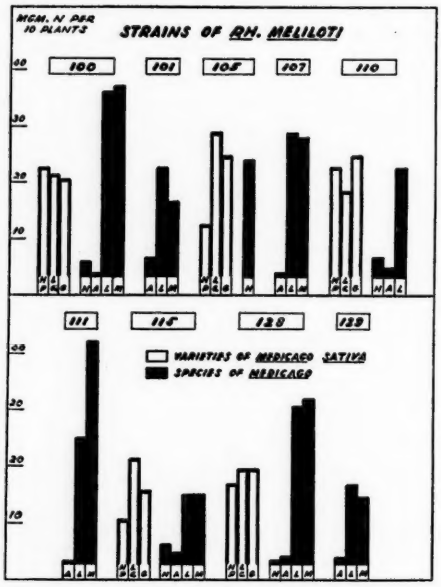


FIG. 2. EFFECT OF STRAIN OF RH. MELILOTI ON NITROGEN FIXATION BY SPECIES OF MEDICAGO
(Experiment 4, August 28 to November 27.)
HP, Hairy Peruvian; G, Grimm; A, *M. arabica;* M, *M. minima;* La, Ladak; H, *M. hispida;* L, *M. lupulina.*

The origin of the variance due to *interaction of strain and species* is readily evident from consideration of the data in table 3. With *M. minima* and *M. lupulina* as the host plants, all strains of the organism would be classed as "good." With *M. sativa* (*Grimm*) all strains except 105 were "good"; this strain was somewhat erratic but, in general, would be classed as "fair." In contrast to their response in association with these three species, all the strains except 105 fixed practically no free nitrogen in association with *M. arabica* and *M. hispida*. The superiority of strain 105 on these two species was quite unexpected, since with host plants previously tested (*M. sativa* and different

species of *Melilotus*) this strain had been usually classed as "poor" to "fair." On the other hand, the remaining strains were definitely "good" on these hosts.

The origin of the variance due to *species* obviously arises from the fact that *M. arabica* and *M. hispida* fix nitrogen only when inoculated with strain 105, whereas the other species fix relatively large quantities with all the strains used. Likewise, the significant variance due to *strain* originates from the circumstance that strain 105 is fair to good on all the species, whereas the other strains are very poor on *M. arabica* and *M. hispida*.

The conclusions reached in these experiments were confirmed by a fourth trial (experiment 4) conducted during the fall growing season. The 2-gallon jars were used with *M. sativa* (three varieties) and *M. hispida* in one set, and with *M. arabica*, *M. lupulina*, and *M. minima* in a second. The five strains of bacteria used in the majority of these experiments furnished the inocula for the plants of the first set. In an effort to discover other strains which could fix nitrogen in association with *M. arabica* and *M. hispida*, the plants of the second set were tested with several strains not previously used with these particular species. The data, which are summarized in figure 2, confirm in all essential respects those of the previous experiments. With the varieties of *M. sativa* there is some evidence of host plant specificity, especially with strains 105 and 115, but the differences are not marked. With the species, however, results identical with those of the first two experiments were obtained. It is noteworthy that none of the previously untested strains was able to fix nitrogen in association with *M. arabica* and *M. hispida*; of the nine strains employed in the four experiments, only 105 was found to be efficient with these two species of the host plant.[5]

SUMMARY

Three varieties of *Medicago sativa* (*Ladak*, *Hairy Peruvian*, and *Grimm*) were tested for ability to fix nitrogen in association with nine strains of *Rhizobium meliloti* under different environmental conditions. Although definite evidence was obtained for the existence of host plant specificity (i.e., fixation of nitrogen with a given strain varying with the variety of host plant), its occurrence tended to be erratic. Apparently, the relationship between the plant and the bacteria is affected by other factors, e.g., environmental conditions, which may obscure the specific influence of the host plant.

Five species of *Medicago* (*M. sativa*, *M. arabica*, *M. hispida*, *M. lupulina*, and *M. minima*) also were tested for nitrogen-fixing ability in association with the same nine strains of the organism. Unequivocal host plant specificity was established among these species. With *M. sativa*, *M. lupulina*, and *M. minima* all strains were very efficient in fixation of nitrogen, with the possible exception of strain 105 in association with *M. sativa*. In contrast, only strain 105 was capable of nitrogen fixation in association with *M. arabica* and *M. hispida*.

[5] Since this manuscript was submitted, we have succeeded in isolating several strains of *Rh. meliloti* which are efficient on *M. arabica* and *M. hispida*.

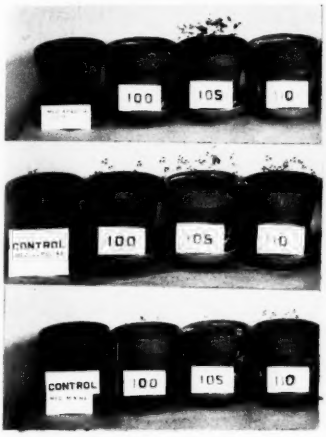
PLATE 1

Host Plant Specificity in Species and Varieties of Medicago

Fig. 1. Growth of three species of *Medicago* on nitrogen-poor sand when inoculated with *Rh. meliloti*, strains 100, 105, and 110. This picture was taken when the plants were 1 month old; with strains 115 and 128 essentially the same effect was obtained as with strains 100 and 110.

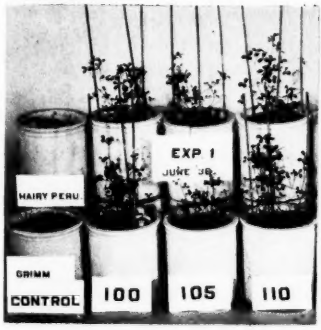
Fig. 2. Roots of *M. arabica* at harvest, showing that invasion of the plant had occurred even though no nitrogen fixation resulted except with strain 105. From left to right: Control, 100, 105, 110, 115, 128.

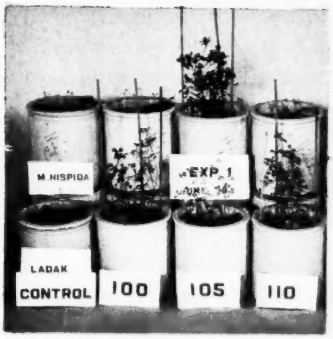
Fig. 3. Growth of *M. hispida* and three varieties of *M. sativa* (*Ladak, Hairy Peruvian, Grimm*) on nitrogen-poor sand when inoculated with *Rh. meliloti* 100, 105, and 110. These plants were of the same series as those of figure 1; the picture was taken at harvest.


100
105
0
CONTROL
100
105
110
CONTROL
100
105
110

1

2


EXP 1
HAIRY PERU
GRIMM
CONTROL
100
105
110


M NISPIDA
LADAK
CONTROL
100
105
110

3

THE AVAILABILITY, TO CROP PLANTS, OF DIFFERENT FORMS OF SELENIUM IN THE SOIL[1]

O. E. OLSON AND A. L. MOXON[2]

South Dakota Agricultural Experiment Station

Received for publication September 26, 1938

The natural occurrence of selenium in the soils of certain areas and the production of toxic vegetation on these soils have resulted in many studies on the absorption of selenium by plants. Early studies in this laboratory indicated that the total selenium content of a soil could not always be used as an index of the selenium content of vegetation growing on the soil. Hurd-Karrer (12, 13, 14) and Byers (7) have reported that the sulfate content of soils has considerable influence on the absorption of selenium by plants. Hurd-Karrer (13, 14) reported on work with selenium salts in the greenhouse in which the addition of sulfur to selenized cultures inhibited the absorption of selenium by the plants. Experiments reported by Franke and Painter (11) showed that the addition of sulfur to field plots in a seleniferous area failed to inhibit the absorption of selenium by crop plants. Beath et al. (4) showed that the addition of sulfur actually increased the absorption of selenium from soils fertilized with highly seleniferous plants. Beath (15) contends that the form of selenium in the soil is of primary importace and that the sulfur-selenium antagonism has been overemphasized. Moxon (16), likewise, has pointed out that the form of selenium is of more practical importance than the sulfur content, since many of the soils producing toxic vegetation are highly saturated with gypsum.

Beath et al. (3) in 1935 reported that certain plants belonging to such genera as *Astragalus*, *Oonopsis*, and *Stanleya* act as converters and make selenium available to other plants.

SELENIUM IN GEOLOGICAL FORMATIONS

The first studies on the selenium problem indicated that the selenium occurred in soils derived from Pierre shales. Later, Beath et al. (2) reported that in Wyoming seleniferous plants were found on virtually all formations of the Cretaceous period, the most toxic plants occurring on the Niobrara, Pierre, and Steele formations. The Niobrara, although limited to rather small areas, is likewise very seleniferous in South Dakota (16).

Most of the seleniferous soil of central South Dakota has been derived from

[1] Approved for publication by the director of the South Dakota Experiment Station and published as a contribution from the experiment station chemistry department of South Dakota State College.

[2] The authors wish to thank Morris Rhian, George Stanford, and Henry Lardy for assistance with the analytical and greenhouse work.

the Pierre formation. Searight (18) has recently divided the Pierre into five members. This division of the Pierre into members, with later modifications by Searight (19), appears in figure 1. A recent study by Moxon, Olson, Searight, and Sandals[3] indicates that the most extensive seleniferous areas in South Dakota are composed of soils derived from the Mobridge member of the Pierre formation. Soils derived from the Sharon Springs member, like soils derived from the Niobrara formation directly below, are also usually highly seleniferous.

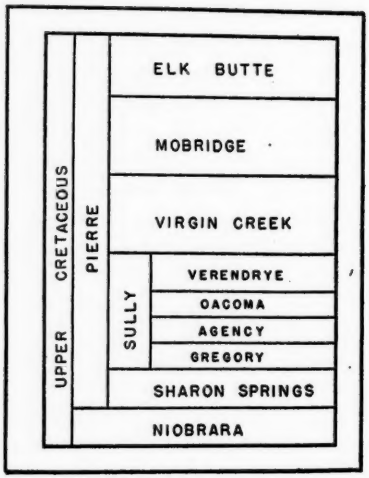


FIG. 1

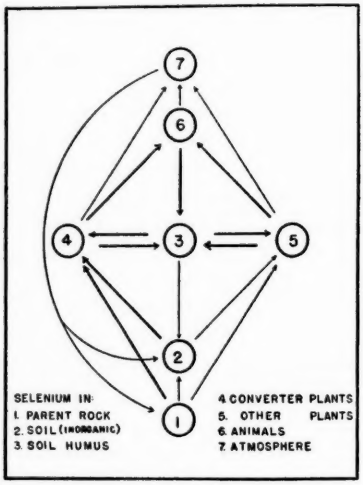


FIG. 2

FIG. 1. MEMBERS OF THE PIERRE FORMATION
According to Searight (18, 19)
FIG. 2. THE SELENIUM CYCLE IN NATURE

FORMS OF SELENIUM IN THE SOILS

Williams and Byers (20) reported that selenium occurs in the soil in at least three forms; namely, basic iron selenite (exact formula undetermined), iron selenide, and a selenate. Byers (8) noted that calcium selenate, and possibly selenite, are present in soils of high water-soluble selenium content, and later (9) that elemental selenium does not occur naturally in soils. Byers (8) has also said, "It appears very certain that but little selenium is associated with the soil organic matter after it has become humified."

The authors are of the opinion that it is the selenium in the organic fraction of the soil which becomes most readily available to plants. This view is in accordance with the theory advanced by Beath et al. (3, 4) regarding the conversion of inorganic selenium to organic selenium by "converter" plants. The selenium cycle in the soil and in plants is rather complicated (fig. 2). After the selenium is once made available to crops by "converter" plants the crop

[3] Unpublished data.

plants are probably able to maintain the cycle for many years, especially if a small amount of the "unavailable" selenium becomes available to the crop plants each year to offset the losses of selenium removed by crops. There is a small loss of selenium from plants and animals into the atmosphere in the form of volatile organic compounds. The selenium in these organic compounds would probably be returned to the soil or geological formations and might be bound with iron compounds to become unavailable.

The organic selenium from decaying vegetation returns to the soil, where most of it remains in the humus fraction. Soil samples taken where there have been heavy growths of highly seleniferous vegetation show high concentrations of selenium in the humus.[4] Selenium in humus evidently occurs in organic form, but the exact chemical nature is not known. Various organic

TABLE 1

Sampling location of soils

SOIL NUMBER	LABORATORY NUMBER	LOCATION
1	1314	Dean Farm. S.E.1/4 Sec. 29 T.3N. R.31E. Stanley Co., S. Dak. Hill N.W. of dam.
2	1315	Dixon Selenium Experimental Plot N.W.1/4 of S.E.1/4 of Sec. 21 T.100N. R.72W. Gregory Co., S. Dak.
3	1316	Reed Farm. S.E.1/4 of N.E.1/4 of Sec. 2 T.107N. R.78W. Lyman Co., S. Dak.
4	1317	Reed Farm. S.W.1/4 of N.W.1/4 of Sec. 2 T.107N. R.78W. Lyman Co., S. Dak.
5	1318	Dean Farm. S.E.1/4 of Sec. 29 T.3N. R.31E. Stanley Co., S. Dak. Hill W. of dam.
6	1319	Reed Farm. S.W.1/4 of S.E.1/4 of Sec. 2 T.107N. R.78W. Lyman Co., S. Dak.

selenium compounds exist in plants. The selenium in cereals is in the protein fraction and is not water soluble (10); on the other hand, some of the selenium in certain converter plants is readily soluble in water.

EXPERIMENTAL

Six soils from different locations in two seleniferous areas, as shown in table 1, were studied to determine the form of selenium present and its relation to availability to crop plants. These soils were all taken from farms reputed to be seleniferous.

Chemical and physical analyses of soils

The soils were well mixed when they were received at the laboratory. Samples sufficient for all analyses were reserved, and the remainder of the soils were placed in plats in the greenhouse for tests to determine the absorption of selenium by vegetation.

[4] Unpublished data from this laboratory.

The soils were analyzed for total nitrogen, total calcium, total iron, and total sulfur as sulfates by the methods of the Association of Official Agricultural Chemists (1). The hydrogen-ion concentrations were determined by the use of a glass electrode. Base-exchange capacity was determined by the "barium acetate-ammonium chloride" method as described by Parker (17). Mechanical analyses of the soils were made by the methods of Bouyoucos (5, 6), and the texture classes were determined according to the U. S. Department of Agriculture method of classification. All selenium analyses were made by the digestion-distillation method as described by Moxon (16).

TABLE 2

Mechanical analyses of soils

SOIL NUMBER	LABORATORY NUMBER	SAND	SILT	CLAY	VERY FINE CLAY	TOTAL COLLOID	TEXTURAL CLASS
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
1	1314	13.6	26.8	59.6	57.0	72.0	Clay
2	1315	12.4	33.0	54.6	51.0	64.6	Clay
3	1316	11.0	21.0	68.0	61.2	73.6	Clay
4	1317	17.6	31.8	50.6	45.2	55.2	Clay
5	1318	10.0	26.8	63.2	55.6	74.0	Clay
6	1319	34.0*	53.8	12.2	11.6	12.8	Silt loam

* High gypsum content.

TABLE 3

Nitrogen, calcium, and iron content, pH, and base exchange of soils

SOIL NUMBER	LABORATORY NUMBER	TOTAL NITROGEN	TOTAL CALCIUM	TOTAL IRON	pH	BASE EXCHANGE
		per cent	*per cent*	*per cent*		*m.e./100 gm.*
1	1314	0.204	7.15	3.670	7.70	28.51
2	1315	0.304	4.08	4.232	7.73	33.49
3	1316	0.224	4.09	4.033	7.73	37.48
4	1317	0.336	2.48	3.836	7.50	38.06
5	1318	0.254	7.81	4.232	7.78	31.73
6	1319	0.151	17.56	2.447	7.20	14.93

Humus determinations were made by leaching the soils with 1 per cent HCl until the leachate was free of calcium and magnesium and then extracting the humus from the soil with 4 per cent NH_4OH. The leachate from the HCl washing was evaporated to dryness and analyzed to determine the amount of acid-soluble selenium. Selenium was also determined on the humus and the evaporated ammonium hydroxide washings as well as on the soil residues remaining after the HCl and NH_4OH leachings. The results of the chemical and physical analyses are shown in tables 2 to 5.

Greenhouse experiments

Crops were grown on the six soils in greenhouse plats to determine the availability of the selenium to plants. Two plantings each of corn, wheat, oats,

and barley were made. One planting of sorghum and one planting of mustard were made. The crops were taken for analysis when they reached a height of 10 or 12 inches. The plants were dried and analyzed for selenium by the

TABLE 4

Sulfur-selenium relationships of soils

SOIL NUMBER	LABORATORY NUMBER	SULFUR AS SULFATES		SELENIUM	
		Total	Soluble	Soluble	Total
		per cent	*per cent*	*p.p.m.*	*p.p.m.*
1	1314	0.248	0.032	0.05	4.8
2	1315	0.455	0.026	0.026	5.7
3	1316	0.320	0.014	Trace	1.7
4	1317	0.455	0.035	<0.026	3.8
5	1318	0.315	0.034	<0.026	6.0
6	1319	24.44	0.789	<0.026	4.2

TABLE 5

Amounts of various forms of selenium in the soils and the relationship to selenium content of plants grown on them

SOIL NUMBER	LABORATORY NUMBER	HUMUS	TOTAL Se	ORGANIC Se	ACID-SOLUBLE SELENIUM	WATER-SOLUBLE SELENIUM	AVERAGE Se CONTENT OF 10 CROPS (DRY BASIS)
		per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	1314	11.3	4.8	2.1	0.33	0.05	27.368
2	1315	11.3	5.7	1.7	0.153	0.026	12.075
3	1316	12.7	1.7	0.42	0.199	Trace	0.15
4	1317	12.7	3.8	1.46	0.067	<0.026	0.65
5	1318	10.7	6.0	1.55	0.100	<0.026	3.205
6	1319	4.0	4.2	0.44	0.078	<0.026	2.552

TABLE 6

Selenium content of the various crops on each soil

p.p.m. dry weight

SOIL NUMBER	LABORATORY NUMBER	WHEAT IA	WHEAT IB	CORN IA	CORN IB	BARLEY IA	BARLEY IC	OATS IA	OATS ID	SORGHUM IB	MUSTARD
1	1314	35.0	28.6	16.3	9.4	28.0	13.8	23.0	29.78	4.8	85.0
2	1315	15.0	6.5	5.6	4.0	20.0	7.1	15.0	9.25	4.3	34.0
3	1316	0	0	0	0	0	1.2	0	0.3	1.0	0
4	1317	0	0	0	0	3.7	0	0	0.2	0	2.6
5	1318	5.0	0	1.1	0	8.0	0	6.0	2.45	0.5	9.0
6	1319	3.5	0	3.4	0.6	9.0	0	4.0	2.42	0	2.6

digestion-distillation method (16). The average selenium content of the 10 crops is shown in the last column of table 5. The selenium content of each crop on each of the six soils is shown in table 6.

CONCLUSIONS

The results of the analytical and greenhouse work presented in the foregoing tables indicate that the availability of selenium to plants depends largely upon the form in which it exists in the soil. The availability of selenium in the soils is apparently directly dependent upon the amount of water-soluble selenium in the soil, which is evidently correlated with or dependent upon the selenium in the organic fraction or humus of the soil. The last column of table 5 shows the average selenium content of 10 crops raised on each soil, and table 6 shows the selenium content of each crop raised on each soil. A comparison of the selenium content of the crops with the amounts of water-soluble selenium and organic selenium in each soil shows a relatively close relationship, whereas a comparison of the selenium content of the crops with total selenium or acid-soluble selenium shows practically no direct correlation.

Table 4 indicates that there is no relationship between the total sulfates or soluble sulfates of the soils studied and the availability of the selenium to the plants grown on them.

SUMMARY

Six soils from seleniferous farms were analyzed for various important constituents including total and water-soluble sulfur and total, water-soluble, acid-soluble, and organic selenium. Ten plantings were made on the soils in the greenhouse, and the plants were analyzed for selenium to determine the availability of the selenium in the six soils to various plants. The availability of the selenium in soils appears to be dependent upon the amount of water-soluble selenium, which in turn seems to be dependent upon or correlated with the amount of organic selenium in the soil. The total sulfur content and the water-soluble sulfur content of a soil appear to be of little or no significance in determining the availability of selenium to plants in a naturally seleniferous soil. The selenium cycle and the forms of selenium in soils are discussed briefly.

REFERENCES

(1) Association of Official Agricultural Chemists 1935 Official and Tentative Methods of Analysis, ed. 4. Washington, D. C.

(2) Beath, O. A., et al. 1934 Certain poisonous plants of Wyoming activated by selenium and their association with respect to soil types. *Jour. Amer. Pharm. Assoc.* 23: 94–97.

(3) Beath, O. A., Eppson, H. F., and Gilbert, C. S. 1935 Selenium and other toxic minerals in soils and vegetation. Wyo. Agr. Exp. Sta. Bul. 206.

(4) Beath, O. A., Eppson, H. F., and Gilbert, C. S. 1937 Selenium distribution in and seasonal variation of type vegetation occurring on seleniferous soils. *Jour. Amer. Pharm. Assoc.* 26: 394–405.

(5) Bouyoucos, G. J. 1927 The hydrometer as a new and rapid method for determining the colloidal content of soils. *Soil Sci.* 23: 319–330.

(6) Bouyoucos, G. J. 1934 A comparison between the pipette method and the hydrometer method for making mechanical analysis of soils. *Soil Sci.* 38: 335–343.

(7) BYERS, H. G. 1935 Selenium occurrence in certain soils in the United States with a discussion of related topics. U. S. Dept. Agr. Tech. Bul. 482.

(8) BYERS, H. G. 1936 Selenium occurrence in certain soils in the United States with a discussion of related topics—Second report. U. S. Dept. Agr. Tech. Bul. 530.

(9) BYERS, H. G., ET AL. 1938 Selenium occurrence in certain soils in the United States with a discussion of related topics—Third report. U. S. Dept. Agr. Tech. Bul 601.

(10) FRANKE, K. W., AND PAINTER, E. P. 1936 Selenium in proteins from toxic foodstuffs. I. Remarks on the occurrence and nature of the selenium present in a number of foodstuffs or their derived products. *Cereal Chem.* 13: 67–70.

(11) FRANKE, K. W., AND PAINTER, E. P. 1937 Effect of sulfur additions on seleniferous soils. *Indus. and Engin. Chem.* 29: 591.

(12) HURD-KARRER, A. M. 1933 Inhibition of selenium injury to wheat plants by sulfur. *Science* (n.s.) 78: 560.

(13) HURD-KARRER, A. M. 1934 Selenium injury to wheat plants and its inhibition by sulfur. *Jour. Agr. Res.* 49: 343–357.

(14) HURD-KARRER, A. M. 1935 Factors affecting the absorption of selenium from soils by plants. *Jour. Agr. Res.* 50: 413–428.

(15) KNIGHT, S. H., AND BEATH, O. A. 1937 The occurrence of selenium and seleniferous vegetation in Wyoming. Wyo. Agr. Exp. Sta. Bul. 221.

(16) MOXON, A. L. 1937 Alkali disease or selenium poisoning. S. Dak. Agr. Exp. Sta. Bul. 311.

(17) PARKER, F. W. 1929 Base exchange capacity. *Jour. Amer. Soc. Agron.* 21: 1030–1039.

(18) SEARIGHT, W. V. 1937 Lithologic stratigraphy of the Pierre formation of the Missouri Valley in South Dakota. S. Dak. State Geol. Survey Rpt. Invest. 27.

(19) SEARIGHT, W. V. 1938 The microfauna of the Sully member of the Pierre. *Proc. Iowa Acad. Sci.* (In press).

(20) WILLIAMS, K. T., AND BYERS, H. G. 1936 Selenium compounds in soils. *Indus. and Engin. Chem.* 28: 912–914.

SOME MOISTURE RELATIONSHIPS OF SOILS FROM BURNED AND UNBURNED LONGLEAF PINE FORESTS

FRANK HEYWARD[1]

Division of Forestry, Department of Natural Resources, Atlanta, Ga.

Received for publication August 29, 1938

Moisture is probably the most important soil factor influencing forest growth in the longleaf pine region. This is true in spite of the comparatively high annual precipitation of 45 to 60 inches throughout the region and the fact that rainless periods exceeding 4 to 5 weeks are rare. Several factors are responsible for this condition. In the first place, within the longleaf pine region the number of days that are warm to hot with intense sunshine is relatively large. This weather is conducive to great evaporation of moisture from free surfaces, an example of which is soil exposed to the atmosphere. Moreover, such weather stimulates a high transpiration rate, which still further tends to deplete soil moisture. Because of this prevalence of days of full sunshine, the rainfall capable of being utilized by forest growth is unquestionably much less than the actual rainfall.[2] Another factor tending to minimize effective rainfall is the sandy nature of a large percentage of pine forest soils. These soils, which commonly vary in texture from fine sand to fine sandy loam, have comparatively low normal moisture capacities. Lastly, either the dense ground cover of perennial grasses or the low underbrush of palmetto and gallberry present in almost all longleaf pine forests, doubtless consumes great quantities of soil moisture.

From the foregoing considerations, it is evident that any factor which might possibly alter soil-moisture conditions in pine forest soils is of much importance in silviculture. One factor which should, therefore, be considered in this respect is fire. Fires, largely of the ground-fire class, are more prevalent in the longleaf pine region than in any other forest region in the country. During past generations the longleaf forest that was unburned for longer than 2 or 3 years was indeed exceptional. Within the last 10 years, however, fire protection has made rapid strides in the region, and it is now possible to find extensive forests protected from fire for 5 or more years.

That frequently burned and long-unburned pine forests differ greatly in floristic and also in certain soil characteristics has been revealed by studies by the Southern Forest Experiment Station. In order to determine whether soil moisture likewise differed, the present study was undertaken.

[1] Formerly assistant silviculturist, Southern Forest Experiment Station.

[2] Data on the rainfall-evaporation ratio are virtually nonexistent for the longleaf pine region.

DESCRIPTION OF STUDY AREAS

Within a 40-mile radius of Lake City, Florida, four areas suited for study were located. At each location were a stand of second-growth longleaf or slash pine protected from fire for several years, and, adjoining the unburned stand, a similar stand which had been exposed to repeated annual fires. Inasmuch as the only feature separating the burned and unburned portions of each study area was either a fence or a ploughed firebreak, conditions of soil and topography in each case were practically identical for both portions. A

TABLE 1

Description of unburned portions of study areas

AREA	FOREST TYPE AND FIRE HISTORY	NATURE OF STAND	SOIL AND DRAINAGE
Newberry, Fla.	Longleaf pine second growth. Unburned since 1928.	Understocked. No forest floor except directly beneath largest trees. Dense ground cover of wire grass. Scattered clumps of dwarf chinquapin and saw palmetto.	Norfolk fine sand. Dry site.
Area I, Olustee, Fla.	Longleaf pine second growth. Unburned since 1929.	Understocked. No forest floor except directly beneath largest trees. Dense ground cover of wire grass. Scattered clumps of saw palmetto.	Leon fine sand. Poorly drained, but soil dries out quickly because of presence of hardpan at a depth of approximately 15 inches.
Area II, Olustee, Fla.	Longleaf pine second growth. Unburned since 1930.	Understocked. No forest floor except directly beneath largest trees. Dense ground cover of wire grass. Scattered gallberry and saw palmetto.	Plummer fine sand. Poorly drained site, continually moist throughout years of normal rainfall.
Raiford, Fla.	Slash-loblolly pine second growth. Unburned since 1924.	Old field stand, practically normally stocked. Forest floor fairly well developed.	Blanton fine sand. Normally a fairly moist site, although the soil dries out rapidly after a rain.

description of the unburned portions of the areas studied is given in table 1. In stand character, burned and unburned areas were closely alike; but certain important differences existed with respect to ground cover, which will be discussed in detail later.

FIELD WORK

The field work of the study was begun in the summer of 1935. Soil samples for moisture determinations were collected from burned and unburned plots from the several locations. For each collection a total of 20 individual samples

per plot was obtained from each of three depths. A sampling tube, type A, described elsewhere (1), was used. Samples collected from 0–2, 4–6, and 8–10 inches were placed in metal cans with tightly fitting covers and transported to the laboratory for immediate moisture determination. Moisture percentages are based on soil heated to constant weight at 100–105°C.

No attempt was made to sample the areas at regular intervals; rather, it was believed preferable to sample according to weather conditions, i.e., during dry or wet periods. For this reason, if one area had been sampled in June when the soil was very moist, no attempt was made to resample the area until the rainy period had passed and soil-moisture conditions had changed markedly. This procedure was followed, rather than that of sampling at regular intervals, because throughout the study rainfall was abundant and periods during which moisture was critical were rare. Sampling at regular intervals, therefore, would have provided a poor distribution of data with respect to field moisture conditions.

Following are data showing the monthly rainfall at Olustee, Florida, during the time of the study:

1935	RAINFALL	1936	RAINFALL
	inches		*inches*
June	5.21	January	4.74
July	14.61	February	6.42
August	10.11	March	4.36
September	7.96	April	1.47
October	0.63	May	2.54
November	0.78	June	3.95
December	1.66	July	5.43

Monthly mean: 4.99 inches.

RESULTS OF DETERMINATIONS OF FIELD MOISTURE PERCENTAGE

The results of the determinations of field moisture percentage are presented in table 2, which shows actual field conditions of soil moisture for four paired burned and unburned areas. The differences are summarized in figure 1 in graphic form according to their statistical significance, i.e., the figure represents the values in the columns headed "Difference divided by standard error of difference," in table 2.

From these data it is clear that to a depth of at least 10 inches the soil of the unburned areas was more moist than that from corresponding depths of frequently burned areas. Regardless of whether field moisture was high or low, the soil protected from fire was, in general, more moist. The high degree of consistency for the 0–2-inch depth is noteworthy; but 2 cases out of a total of 28 showed more moisture in the soil exposed to fire. It will be noted in table 2 that although the actual difference in percentage of field moisture was relatively small (varying from less than 1 to slightly less than 10), the percentage differ-

TABLE 2

Field moisture percentages of soils from burned and unburned longleaf pine forests

DATE COLLECTED	AT 0–2 INCHES				AT 4–6 INCHES				AT 8–10 INCHES			
	Unburned mean	Burned mean	Percentage difference	Difference divided by standard error of difference	Unburned mean	Burned mean	Percentage difference	Difference divided by standard error of difference	Unburned mean	Burned mean	Percentage difference	Difference divided by standard error of difference
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	
Newberry												
Jul. 17, 1935.....	10.59	11.04	4.2	0.75	7.49	7.76	3.6	0.79	7.33	8.30	13.2	1.49
Aug. 19, 1935.....	8.62	9.00	4.4	0.68	6.59	6.90	4.7	1.53	6.59	7.03	6.7	1.77
Sept. 6, 1935.....	12.98	11.94	8.0	−1.82	8.56	8.63	0.8	0.17	7.35	7.96	8.3	1.74
Oct. 23, 1935.....	3.40	2.56	24.7	−2.16*	2.55	2.42	5.1	−0.64	2.05	1.96	4.4	−0.51
Dec. 17, 1935.....	8.97	8.90	0.8	−0.13	7.00	6.96	0.6	−0.18	6.72	6.88	2.4	0.74
Apr. 7, 1936.....	8.57	7.50	12.5	−1.67	6.15	6.36	3.4	0.41	6.12	5.79	5.4	−1.05
May 11, 1936.....	1.60	1.24	22.5	−1.94	1.78	1.44	19.1	−2.45*	1.70	1.40	17.6	−2.34*
Jul. 9, 1936.....	3.72	2.12	43.0	−3.96*	3.08	2.28	26.0	−2.94*	2.28	1.74	23.7	−2.48*
Raiford												
Jul. 4, 1935.....	4.87	2.74	43.7	−4.04*	4.37	3.31	24.2	−3.46*	4.13	3.03	26.6	−4.64*
Jul. 31, 1935.....	21.64	17.94	17.1	−0.98	14.58	15.46	5.9	0.31	13.75	12.73	7.4	−0.37
Aug. 26, 1935.....	15.32	14.05	8.3	−0.89	9.92	9.46	4.6	−1.27	9.12	9.02	1.0	−0.21
Sept. 25, 1935.....	12.72	12.08	5.0	−1.00	10.08	9.18	8.9	−1.68	10.09	9.54	5.5	−0.95
Oct. 18, 1935.....	7.04	3.94	44.0	−4.89*	5.18	4.65	10.2	−1.15	6.10	5.21	14.6	−2.35*
Dec. 4, 1935.....	3.91	3.52	10.0	−0.91	3.31	2.44	26.3	−3.22*	2.91	2.34	19.6	−2.66*
Mar. 3, 1936.....	9.34	8.16	12.6	−2.15*	7.73	6.78	12.3	−3.04*	7.09	6.83	3.7	−1.20
May 14, 1936.....	1.67	0.93	44.3	−3.56*	2.81	2.11	25.3	−3.20*	2.52	2.23	11.5	−1.44

Olustee I (sec. 33)

Aug. 5, 1935.....	30.82	28.07	8.9	−1.48	17.82	17.90	0.4	0.11	19.52	18.85	3.4	−0.71
Nov. 4, 1935.....	19.77	18.80	4.9	−0.68	6.86	7.54	9.9	1.13	8.42	9.07	7.7	0.93
Jan. 6, 1936.....	24.22	23.43	3.3	−0.44	9.12	9.67	6.0	0.68	9.76	10.38	6.4	1.18
Feb. 18, 1936.....	34.70	34.69	0.0	−0.005	21.28	21.21	0.3	−0.08	20.20	20.01	0.9	−0.15
Apr. 22, 1936.....	25.86	23.23	10.2	−1.24	10.51	10.39	1.1	−0.19	12.36	12.08	2.3	−0.35
Jul. 3, 1936.....	10.05	7.13	29.1	−4.13*	5.62	5.46	2.8	−0.23	6.60	6.36	3.6	−0.36

Olustee II (sec. 18)

Aug. 12, 1935.....	11.78	10.11	14.2	−1.74	7.50	6.70	6.7	−0.63	12.95	5.51	57.5	−8.40*
Oct. 3, 1935.....	24.48	18.58	24.1	−4.90*	21.36	15.75	26.3	−4.41*	23.00	19.78	14.0	−4.92*
Nov. 15, 1935.....	7.65	4.96	35.2	−3.63*	4.19	2.12	49.4	−7.34*	8.21	3.19	61.1	−5.76*
Jan. 9, 1936.....	13.51	11.62	14.0	−2.37*	6.57	5.65	14.0	−2.73*	10.06	5.72	43.1	−4.97*
Apr. 14, 1936.....	18.69	12.78	31.6	−3.70*	14.00	8.42	40.0	−6.09*	21.57	11.98	44.5	−10.29*
Jul. 6, 1936.....	2.75	1.33	51.6	−4.14*	3.22	1.58	50.9	−3.91*	7.71	2.61	66.1	−5.56*

* Denotes a significant difference, i.e., a ratio greater than 2. A minus sign (−) indicates a burned mean less than the unburned mean.

ence varied up to 66.1 per cent. Thus, for the entries for 0–2 inches of July 6 for Olustee II, whereas the difference was only 1.42 per cent, actually the unburned value was 51.6 per cent greater than the burned.

Although table 2 shows definitely that, throughout the year, soils of unburned areas were more moist than soils exposed to annual fires, it does not explain why the difference existed. As a further means, therefore, of evaluating the data on field moisture percentage, the following additional studies were made.

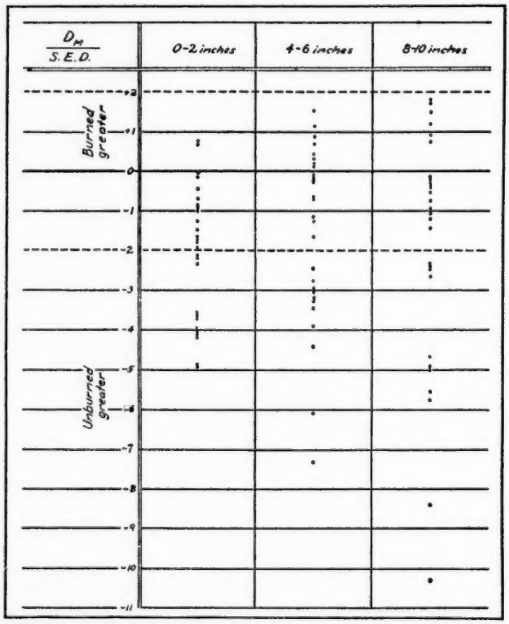


FIG. 1. SOIL MOISTURE PERCENTAGES OF BURNED AND UNBURNED PLOTS IN TERMS OF DIFFERENCE BETWEEN MEANS IN RELATION TO STANDARD ERROR OF DIFFERENCE, WITH SIGNIFICANCE OF RESULTS INDICATED

$D_M = M_1$ (burned mean)—M_2 (unburned mean)
If M_1 is greater than M_2, D_M is shown as +;
If M_1 is less than M_2, D_M is shown as −.
Where $\frac{D_M}{S.E.D.}$ exceeds 2, the difference is statistically significant.

MEASUREMENTS OF MOISTURE RETENTION OF SOILS FROM BURNED AND UNBURNED AREAS

Conceivably, fire may influence moisture relationships of forest soils in two distinct manners; namely, by alterations in the forest floor or vegetative cover, and by possible changes in the soil itself which might affect moisture retention.

A gross measure of the combined effect of all changes associated with fire is readily afforded by direct determinations of field moisture percentage. Although representing the most significant determination within the scope of this study, field moisture determinations alone offer no means of evaluating the specific effect of either of the aforementioned factors. In order to establish net relationships between field moisture percentage and both the factors of vegetative cover and moisture retention of the soil, an experiment was performed, in which several different but related measures of moisture retention were made in the laboratory. The factor of differences in forest floor and vegetative cover being eliminated by experimental design, net results were obtained of the ability of the soil itself to retain moisture.

Tests were made for normal moisture capacity[3] and wilting percentage on the 0–4-inch depth. By means of a specially designed soil tube (1), soil samples were collected in pint cardboard containers in undisturbed field condition. The tests were performed by soaking for 24 hours in distilled water the soil column within the pint carton and then draining for 6 weeks on pure fine white sand of approximately 5 per cent moisture content. Provisions were made to eliminate evaporation. After draining, moisture percentage was determined on half the mixed sample, as usual, and the result was expressed as normal moisture capacity; the remainder of the sample was saved. Twenty samples per treatment per study area were used. Wilting percentage was obtained by the direct method, corn being used as an indicator.

One further test was made. In order to determine whether any differences found in favor of the soil from the burned or from the unburned plot might be attributed either to changes in the nature of the soil particles[4] or to changes in soil structure, determinations were made of the water retained by sifted soil material. Any difference between the two classes of soils studied as shown by this test would be attributable to the effect of changes in soil particles, since the factor of structure was removed by the sifting process. This test was performed as follows: The half of the sample not subjected to oven drying in the determination of normal moisture capacity was passed through a 2-mm. sieve and then placed in a 4-inch funnel provided with rapid filter paper. The soil sample was thoroughly saturated with water and allowed to drain 10 minutes, after which time, the moisture percentage was determined.

The value of the tests of moisture retention would have been enhanced greatly if these tests could have been made on samples from the same depths as those reported in table 2. This was impossible, because of the heavy distribution of roots in the 0–3-inch depth. Since these roots necessitated

[3] Shaw (4) defined normal moisture-holding capacity as "the minimum amount of water that is retained by absorption and film forces when the water is free to move downward through a mass of uniform soil."

[4] By changes in the nature of soil particles is meant possible alterations in soil organic matter and in the colloidal fraction. Also to be considered is the addition to the soil of appreciable quantities of charcoal as a result of fire. The charcoal fragments vary in size from an inch to that of colloidal particles.

taking a soil sample to a depth sufficient to include them, pint containers 4 inches deep were used.

Results of the water retention studies are presented in table 3. Data from this table show that neither large nor consistent differences exist between the two classes of soils in their respective abilities to retain moisture, as measured by wilting percentage and normal moisture capacity. The soil exposed to fire on the Newberry area showed a significantly higher normal moisture capacity than did the soil protected from fire. The opposite condition was true on the Olustee Area II. At Raiford, the soil of the unburned area showed

TABLE 3

Measurements of moisture retention for the 0–4-inch depth by soils from burned and unburned longleaf pine forests

	UNBURNED MEAN	BURNED MEAN	PERCENTAGE DIFFERENCE BETWEEN MEANS	ACTUAL DIFFERENCE BETWEEN MEANS	STANDARD ERROR OF DIFFERENCE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Wilting percentage (undisturbed soil)					
Newberry	2.06	2.11	2.4	0.05	0.182
Olustee, II	1.89	1.87	1.1	0.02	0.178
Raiford	1.96	1.42	27.5	0.54*	0.159
Normal moisture capacity (undisturbed soil)					
Newberry	10.74	11.22	4.5	0.48*	0.161
Olustee, II	12.10	10.78	10.9	1.32*	0.645
Raiford	10.89	10.55	3.1	0.34	0.502
Water-holding capacity (sifted soil)					
Newberry	33.74	32.84	2.7	0.90	0.741
Olustee, II	40.52	39.45	2.6	1.07	1.031
Raiford	41.27	40.82	1.1	0.55	0.494

* Denotes significant difference.

a significantly higher wilting percentage. The results of wilting percentage and normal moisture capacity as obtained for undisturbed soil columns are, therefore, too erratic to be conclusive.

Although the tests of water-holding capacity, as determined on sifted soil, show that there is a slight but consistent tendency for the soils from unburned areas to retain more moisture than the soils exposed to fires, none of the differences indicated in table 3 appear adequate to explain the large and consistent differences in field moisture percentage as given in table 2. Although a mean of only 20 individual samples, the basis of each entry in table 3, offers no high degree of accuracy, it would seem that field sampling was sufficiently intensive

to reveal any large differences in moisture retention, if such existed. That no such large differences were brought out strongly indicates that the sizeable differences in field-moisture percentage are not attributable to the factors measured in table 3, i.e., soil structure and soil composition. These differences must, therefore, be attributed to the third factor capable of influencing moisture, namely, differences in vegetative cover.

Since changes in the forest floor and vegetative cover brought about by fire in longleaf pine forests have been described in considerable detail elsewhere (2, 3), a brief description of prevailing conditions will suffice. Under open canopies in longleaf pine forests protected from fire (pl. 1, fig. 1), a dense ground cover consisting largely of perennial grasses occurs. Low woody vegetation such as gallberry [*Ilex glabra* (L) A. Gray], runner oak [*Quercus minima* (Sarg.) Small.], and saw palmetto [*Serenoa serrulata* (Michx.) Hook.] may be present. The heavy growth of tangled grasses, the leaves of which may be nearly 3 feet long, form a loose mulch over the soil (pl. 2, fig. 1). Much of this material is dead. Under closed stands of pine protected from fire for 10 or more years, little or no herbaceous ground cover occurs, the rapidly accumulating forest litter having effectively smothered out this type of vegetation.

On areas subjected to recurrent fires, however, conditions are vastly different. Regardless of stand density, a luxuriant ground cover exists, consisting of a wide variety of broadleaved herbaceous plants in addition to perennial grasses (pl. 2, fig. 2). The same low underbrush may occur as on unburned areas, but no well-defined forest floor exists, the pine litter being annually consumed by fire. The individual plants comprising the ground cover, at least the aerial portions, are consequently younger than those on unburned areas.

The differences in ground cover are believed to account for the sizeable differences between the moisture content of soil from burned and unburned areas. The data presented indicate that the dense, tangled growth consisting largely of grasses over 7 years of age conserves soil moisture better than does the young, scattered growth typical of areas burned over at frequent intervals. Also, on long-unburned areas, the interwoven mass of living and dead grasses, which forms a good mulch over the soil, contrasts strikingly with the scattered ground cover that leaves exposed much of the mineral soil of annually burned areas. Such a mulch would prevent at least the top 2 inches of soil from drying rapidly.

Probably the strongest support to the theory advanced above is furnished by the Olustee Area I, where the fire history of the plots studied is as follows: The entire area was subjected to repeated fires prior to 1929, but was protected from 1930 to 1933. In the winter of 1933 one portion was burned and has been burned annually since, whereas the adjoining portion has remained unburned. In 1935 (the year the present study was begun), therefore, a 5-year rough existed on the unburned plot as compared with a 1-year rough on the burned.

In view of the data on moisture retention obtained for burned and unburned areas elsewhere in the longleaf pine region,[5] it seems highly improbable that the slight difference in fire history of these two plots could be accompanied by measurable differences between moisture retention as reflected by tests for either wilting percentage or normal moisture capacity.[6] The consistent differences between field moisture percentages are, therefore, attributed to differences between ground cover, i.e., a 5-year rough versus a 1-year rough.

Because of the excellent conditions for plant growth in the longleaf pine region, growth of herbaceous vegetation following fire is extremely rapid. Although most of the fires in the region occur during winter, by early spring the soil supports a pronounced ground cover usually at least a foot high. In spite of the rapid development of the ground cover, however, the soil is exposed in many spots (pl. 2, fig. 2), and doubtless much loss of moisture from shallow depths takes place because of surface evaporation. Numerous observations throughout the region indicate that a grass rough may increase in density for 5 or 6 years following fire, and the soil surface on all except the poorest sites is generally well covered by herbaceous plants by the end of the second growing season. After 5 or 6 years, when a great quantity of grass exists, the living and dead portions being interwoven and tangled to afford a good mulch over the soil, no apparent increase in density of rough takes place. This means that the data for field-moisture percentage (table 2) probably represent maximum differences to be expected between grassy areas of pine forest when one area has been afforded complete fire protection and the other area has been burned over annually. As shown in table 1, all the stands except the one at Raiford were good examples of open stands with dense, herbaceous ground cover. Under closed stands, in which the forest floor has smothered out the ground cover (pl. 1, fig. 2), much greater differences in field moisture percentage than those reported here could probably be expected, not only because under such conditions surface evaporation would be even less than under a dense grass rough, but more particularly because there would be no ground cover to deplete soil moisture through transpiration. The conserving effect of a mulch of organic matter, such as that provided by a well-developed forest floor, on soil moisture near the surface is too well recognized to need further corroboration.

Because of the difficulty of locating long-unburned areas suitable for study, it is not now possible to present data on soil moisture conditions under closed stands of burned and unburned longleaf pine. The stand at Raiford was much denser than any of the others, but this is an old-field stand, and although the forest floor is fairly well established on the unburned plot, the sparse ground cover of the burned plot (though typical of old fields supporting pine forests) is in no way comparable with natural, rough woods.

[5] To be published.

[6] Plans to determine this point in the laboratory were unfortunately interrupted by a change in the author's employment.

As regards the physiological importance of the moisture differences between burned and unburned forests under field conditions as shown above, it should be pointed out that studies made by the author have revealed that the majority of the small roots of longleaf pine are located within the top 2 inches of soil. Moreover, although a mean monthly rainfall of 4.99 inches occurred during the time the present study was conducted, there occurred three periods during which soil moisture approached a critical point, at least for dry sites. As seen from table 2, in October 1935 and in May and July 1936, the soil moisture on the Newberry area was either close to or actually below the wilting percentage, approximately 2.1 per cent (table 3). Obviously, under such dry conditions an absolute difference of only 1 per cent moisture may actually mean that one soil is 30 to 50 per cent moister than another. That such differences in soil moisture are of importance to forest growth, there can be little doubt.

SUMMARY

Under four paired burned and unburned longleaf pine forests a study was made of field moisture percentage throughout the year. In addition, measurements were made of the normal moisture capacity and wilting percentage of undisturbed soil columns and of the water-holding capacity of sifted soil.

All determinations of field moisture percentage of the four areas being grouped, the following odds were obtained: For the 0- to 2-inch depth, in 26 of 28 determinations the soil from the unburned timber stands was more moist, 12 of these differences being statistically significant; for the 4- to 6-inch depth, soils from unburned stands were more moist in 20 of 28 determinations, of which 11 were significant; and for the 8- to 10-inch depth, soils from unburned stands were more moist in 22 of 28 determinations, of which 11 were significant. Although the differences in percentage were small when expressed as *absolute* values, in *relative* values soils from the unburned areas were as much as 52 per cent moister for the 0- to 2-inch depth than for the corresponding soil depth on burned areas.

Differences between moisture retention as measured by wilting percentage and normal moisture capacity obtained for undisturbed soil columns from the 0- to 3-inch depth were neither large nor consistent. The soils protected from fire showed a slightly higher retention of water as determined for sifted soil from the 0- to 3-inch depth. Since the differences in water retention were not sufficiently large to explain the differences found between field moisture percentages, it is concluded that the differences in field moisture were caused by some factor other than the nature of the soil itself.

On the areas protected from fire, occurred a thick interwoven mass of perennial grasses, much of which was composed of dead plant material. This mass formed a continuous loose mulch, which in places was more than 1 foot deep over the soil. Annually burned areas, on the other hand, were characterized by a much sparser ground cover, consisting of vigorously growing plants less than 1 year old. On burned areas the bare soil was exposed in many spots.

The differences in moisture utilization and mulching effects between the two classes of ground cover are believed to be responsible for the higher percentage of soil moisture in longleaf pine forests protected from fire as compared with similar forests subjected to annual fires.

REFERENCES

(1) HEYWARD, F. 1936 Soil sampling tubes for shallow depths. *Soil Sci.* 41: 357–360.
(2) HEYWARD, F. 1937 The effect of frequent fires on profile development of longleaf pine forest soils. *Jour. Forestry* 35: 23–27.
(3) HEYWARD, F., AND BARNETTE, R. M. 1936 Field characteristics and partial chemical analyses of the humus layer of longleaf pine forest soils. Fla. Agr. Exp. Sta. Bul. 302.
(4) SHAW, C. F. 1927 The normal moisture capacity of soils. *Soil Sci.* 23: 303–317.

PLATE 1

FIG. 1. Typical open-grown stand of second-growth longleaf pine. Note the luxuriant ground cover. The low density of the stand makes development of a forest floor extremely slow. In stands of this nature, the herbaceous vegetation persists for years following complete fire protection.

FIG. 2. Forest floor under closed stand of second-growth longleaf pine. The vigorous ground cover of wire grass has been almost completely smothered out except under openings in the canopy (an example of which is seen in the background), where it may persist indefinitely. The area illustrated had been protected from fire for 12 to 15 years when photographed.

FIG. 1

FIG. 2

PLATE 2

FIG. 1. Dense ground cover typical of long-unburned areas. Following 5 to 7 years' protection from fire, no appreciable increase in density of rough occurs.

FIG. 2. Typical view of annually burned areas, photographed approximately 2 months after a fire. The ground cover at this stage of development is insufficiently dense to protect the soil from surface evaporation. The soil, particularly on dry sites, may remain partly bare well into the second growing season following fire.

FIG. 1

FIG. 2

A NEW SPECIES OF AZOTOBACTER[1]

ROBERT L. STARKEY AND P. K. DE[2]

New Jersey Agricultural Experiment Station and Dacca University, India

Received for publication September 23, 1938

The specific, aerobic, nitrogen-fixing bacteria obtained from soil and water can be identified as representatives of a small group of well-described species of *Azotobacter*. These include the first isolated species described by Beijerinck in 1901 (1) as *Azotobacter chroococcum* obtained from soil and *Az. agilis* isolated from canal water. J. G. Lipman described a new species, *Az. vinelandii*, in 1903 (12) and an additional one, *Az. beijerinckii*, in 1904 (13). Both of these were isolated from soil. Other cultures have been named, including the *Az. woodstownii* of J. G. Lipman (13), *Az. vitreum* of Löhnis and Westermann (16), and *Az. smyrnii* of C. B. Lipman and Burgess (11), but information concerning these three organisms is scant, and recent workers do not seem to have recognized them among the cultures that they have isolated.

The present report is concerned with cultures isolated from some soils of India. The cultures have some characteristics which are common to other species of *Azotobacter* but differ in other respects from any of the species which have yet been described. No significant differences were noted between the newly isolated cultures, and they are described as a single new species of *Azotobacter*.

EXPERIMENTAL

In the course of an investigation by one of the authors (2, 3) on the fixation of nitrogen in rice soils of India, it was observed that a fairly large amount of nitrogen was fixed in some soils which did not contain any of the common species of *Azotobacter*. Furthermore, *Clostridium pastorianum* was not present in abundance. Some of these soils were distinctly acid in reaction.

Such observations suggested that some nitrogen-fixing organism which could not be readily recovered by use of the common mannitol medium might be present in these soils. Accordingly, several rice soils from different parts of India were examined for the presence of new nitrogen-fixing bacteria, a

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[2] The cultures were isolated by the second-named author while studying nitrogen fixation in soils of India. Information concerning the distinguishing characteristics was obtained by the first-named author at the New Jersey Agricultural Experiment Station.

One of the authors (P. K. D.) acknowledges with appreciation financial support from the Imperial Council of Agricultural Research in India during the course of these investigations.

somewhat modified medium being used. Different species of *Azotobacter* were isolated, but only one appeared to be new. This organism was detected in only two soils. It occurred abundantly in Dacca soil which had a pH of 4.9, and small numbers were found in a soil from Insein, Burma, of pH 5.2.

Isolation

Silica gel plates were prepared in much the same way as that recommended by Winogradsky (20), the principal difference being that sucrose was used as the source of energy. Each 9-cm. silica gel plate was impregnated with 5 cc. of the following stock solution: K_2HPO_4—0.5 gm., $MgSO_4 \cdot 7H_2O$—0.2 gm., $MnSO_4 \cdot 2H_2O$—trace, $FeCl_3 \cdot 6H_2O$—trace, sucrose—10.0 gm., $CaCO_3$—5.0 gm., water—100 cc.

Small particles of soil were scattered over the surface of the gel, and the plates were incubated at 30°C. No growth was observed for 2 weeks, but after this time, small, white, highly tenacious colonies appeared about the soil particles (fig. 1). The colonies gradually increased in size, and, after a month, some of them became reddish in color. For purification, the organism was grown several times on fresh silica gel plates and then plated on agar medium containing the same nutrients used in the silica gel. Well-isolated colonies were readily obtained from streaks on agar plates and from plates prepared from agar in which dilute cell suspensions were mixed (figs. 2 and 3).

For most of the cultural studies, media were used which had the following composition:

Distilled water	1,000 cc.
Organic material	15.0 gm.
K_2HPO_4	0.8 gm.
KH_2PO_4	0.2 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
NaCl	0.2 gm.
$CaCl_2 \cdot 2H_2O$	0.05 gm.
$Na_2MoO_4 \cdot 2H_2O$	0.0005 gm.
$MnSO_4 \cdot 2H_2O$	0.0005 gm.
$Na_2WO_4 \cdot 2H_2O$	0.0005 gm.
$FeSO_4 \cdot 7H_2O$	0.025 gm.

The organic materials (sucrose, glucose, mannitol, and dextrin) were sterilized separately. In some cases $CaCO_3$ (0.5 per cent) was used in the media in place of the calcium chloride, but the organism grew much better in the absence of calcium carbonate. In some cases it failed to grow in liquid media where $CaCO_3$ was present but developed well in similar media without $CaCO_3$. For solid media, 1.5 per cent of agar was added.

On agar plates with sucrose or glucose, the surface colonies were colorless, round, very much raised, and uniformly turbid, having much the appearance of heavy starch paste. The cells were imbedded in an abundance of slimy material which formed long strings when drawn out with a transfer loop and

which had somewhat elastic properties. In many instances, young colonies had much slime, forming gummy masses from which a portion could be removed only with difficulty. Colonies imbedded in the agar were lens-shaped. Colonies growing below the agar at the region of contact of glass and agar were thin, white, and spreading.

The organism grew very slowly, seldom making any visible growth in less than 3 or 4 days at 30°C. The colonies then developed rapidly and formed the characteristic mucoid masses which fused when they were not well separated. This was particularly the case on streaked plates (fig. 2).

On slants of agar containing glucose or sucrose, the culture formed similar raised, starchy, mucoid material which spread over the entire surface. During prolonged incubation (3 to 4 weeks), a considerable amount of the material settled to the bottom of the slant. A buff to light-brown color appears in old cultures, but this is seldom observed in less than 2 weeks.

Development on various media

The organism grew well on both sucrose and glucose but made no development on dextrin. It grew very slowly on mannitol, making very limited development in even 2 weeks.

No growth appeared on plates of nutrient agar with which diluted suspensions of the organism were mixed before the plates were poured, or on streaked plates of this medium or peptone agar, even with heavy inoculation. Peptone agar containing 0.5 per cent glucose supported limited grayish growth; there was somewhat less development on peptone agar with 2 per cent glucose. Growth was abundant on an agar medium prepared from dilute malt extract, but a strong concentration depressed development. Growth was fairly good on agar prepared from tomato juice and peptone and on agar containing yeast extract and glucose. Thus the organism resembles other species of *Azotobacter* in its inability to grow well on media containing organic nitrogen but no carbohydrate.

Morphology of the cells

Both young and old cultures on various media were examined microscopically. On the carbohydrate media, the cells are very characteristic in appearance and quite unlike the descriptions and pictures of any of the species of *Azotobacter* which have come to the attention of the authors. The cells are relatively large and oval, varying in size from 0.5 to 1.2 by 1.7 to 2.7μ. Their distinctive characteristic is the presence of two, large, highly refractive, spherical bodies in the cells, one at each end (figs. 4 and 5); occasionally only one such body is present. A large part of the cell, generally more than one-half of the total volume, is occupied by these bodies. The globules are somewhat variable in size but are constant in occurrence. They appear not only in young cells but in the cells of cultures incubated for many weeks or even months.

When the organism is cultivated on media containing carbohydrate, there

is little evidence of change in the appearance of the cells over a period of several months. In old cultures occasional cells are spherical and triangular in shape. The globules in cells of very old cultures are not so readily distinguished from the rest of the cell as are the highly refractive globules in young cultures.

The globules did not stain with methylene blue, methyl violet, carbol-fuchsin, safranin, or rose bengal. None of the descriptions and photographs of *Azotobacter* in the papers of Beijerinck (1), Den Dooren de Jong (5), Löhnis and Westermann (16), Löhnis and Hanzawa (15), Löhnis (14), Lipman and Burgess (11), Jones (7), Stapp (19), Kluyver and Van Reenen (9), Lewis (10), Winogradsky (21), or others give any indication that these observers had encountered cultures the cells of which regularly contained large terminal globules. An examination of stock cultures of *Az. chroococcum*, *Az. vinelandii*, and *Az. beijerinckii* revealed cells with irregularly scattered small granules, commonly noted in *Azotobacter*, but no indication of the regular occurrence of a large globule at each end of the cell.

It was conceivable that the globule was fatty material, for Stapp (19) reported that fat is the principal reserve material in *Azotobacter*. He was able to remove about 20 per cent of the dry bacterial mass with ether and chloroform. He also found volutin in *Azotobacter* cells. Recently, Lewis also found both fat and volutin in *Azotobacter* (10). He found that the granules which stained intensely with aqueous solutions of aniline dyes were volutin, whereas the large refractive granules which failed to stain with these dyes were fatty substance.

Various reagents were used to ascertain the nature of the granules in the new *Azotobacter*.[3] When treated with dilute iodine solution, the globules are light yellow, indicating that they are neither iogen nor glycogen (17). Since they fail to stain with aniline dyes, they are not volutin. When the cells were treated with a solution of Sudan III in alcohol and glycerin, the globules became uniformly salmon-colored. Best results were obtained when the cell material was treated with acid to hydrolyze the slime before being stained. About 0.5 cc. of culture material from an agar slant was suspended in 2 cc. of 2 per cent HCl and heated slowly to the boiling point. This caused the slime to disappear and yielded a uniformly turbid watery suspension of cells whose globules stained much more clearly with Sudan III than those of the untreated cells.

The globules also stained with α-naphthol blue, recommended by Dietrich and Liebermeister (4) and Eisenberg (6) for staining fat bodies. The cells taken directly from the slant stained distinctly, but the globules were colored much darker when the capsular material was hydrolyzed with HCl previous to being stained.

When the cells were treated with nile-blue-sulfate, as recommended by Eisenberg (6), the globules took the color characteristic of fat. Cell material

[3] The authors are very much indebted to Florence Tenney for making the various stained preparations and for determining the nature of these globules.

used directly from the slant stained light pink, but when the slime was hydrolyzed before staining, the globules assumed a bright red color. From the results with these various staining reagents, it seems evident that the large globules in these *Azotobacter* cells contain fatty material.

The cells were distinctly Gram-negative. There was no evidence of cysts or spores either in stained or in unstained preparations. Motile cells were seen in cultures 5 to 7 days old, developing upon sucrose and glucose at 30°C. Cells with the typical terminal globules moved about with a smooth gliding

TABLE 1

Nitrogen fixation in liquid media

CULTURE	TOTAL NITROGEN FIXED*	
	38 days	51 days
	mgm.	*mgm.*
Glucose medium		
1	3.4	5.2
2	3.4	4.8
3	2.8	3.1
4	2.1	4.1
Sucrose medium		
1	7.6	9.0
2	6.9	8.6
3	4.8	6.8
4	4.7	7.0
Dextrin medium		
1	0.6	0.4
2	0.5	0.7
3	0.7	
3	13.1†	
4	0.7	0.6

* Nitrogen content of three control solutions averaged 0.30 mgm. N (0.30, 0.20, 0.40).

† Fungus contaminant; very thick, like starch paste. It is probable that the fungus hydrolyzed the dextrin to materials available to the bacterium.

motion. Attempts to stain the flagella met with little success, but an occasional cell was seen with a single polar flagellum.

Cells from media containing peptone differed considerably from those developing in the nonnitrogenous media containing carbohydrate. On peptone agar containing 0.5 or 2.0 per cent glucose, there were some typical cells but a predominance of unusually large, swollen, yeastlike cells. Even these generally contained fairly large globules. Similar round, elongated, and irregular cells appeared on tomato agar; some cells seemed to have buds. On malt agar and

on agar containing yeast extract and glucose, most of the cells were the typical rods with terminal globules. The production of abnormally shaped cells by *Azotobacter* in the presence of nitrogenous material has been noted frequently, probably most recently by Den Dooren de Jong (5) and Winogradsky (21).

Nitrogen fixation

The cultures were originally tested for nitrogen-fixing capacity by the use of silica gel plates (20 cm. in diameter) containing the mineral nutrients with 1 gm. of sucrose, such as was used in the isolation experiments. During incubation for 4 weeks, the amounts of nitrogen fixed varied between 7.0 and 9.8 mgm. per plate.

In subsequent tests for nitrogen fixation the more complete liquid medium was used in 100-cc. quantities in 500-cc. Erlenmeyer flasks. For the first tests, $CaCO_3$ was added to the liquid just before inoculation. Four cultures were used: numbers 1 and 2 were isolations from Dacca soil; numbers 3 and 4 were from the soil of Insein, Burma. As shown in table 1, the organisms grew and fixed considerable amounts of nitrogen on both sucrose and glucose.

TABLE 2

*Fixation of nitrogen on silica gel plates**

ORGANISM	PLATE 1	PLATE 2	PLATE 3	AVERAGE
Az. chroococcum	8.9	9.5	10.1	9.5
Az. vinelandii	11.9	11.2	....	11.6
Culture 2	8.0	5.1	5.1	6.1

* Mgm. nitrogen fixed per plate containing 1.5 gm. glucose.
Controls (6) contained from 0.5 to 0.8 mgm., with average of 0.7 mgm.

Maximum fixation was not obtained in 38 days. In 51 days, the nitrogen fixed per flask varied between 3.1 and 9.0 mgm., being nearly twice as great on sucrose as on glucose. The liquid became turbid in about 3 weeks, and some sediment accumulated, but there was no surface membrane. Even after 2 months, there was scarcely any growth in the dextrin solution. The slight increases in nitrogen over the controls do not appear to be significant.

Culture 2 was also grown on silica gel plates (14 cm. in diameter) containing the same amount of nutrients which were present in the 100-cc. portions of liquid media; glucose was the source of energy. For comparison, cultures of *Az. chroococcum* and *Az. vinelandii* were cultivated under similar conditions. *Az. chroococcum* produced an abundance of brown growth. *Az. vinelandii* and culture 2 formed buff-colored growth. Because of the large amount of slimy material produced, culture 2 appeared to form much more cell material than the other two. As shown by the results of nitrogen determinations made after incubation for 28 days (table 2), culture 2 actually fixed less nitrogen than did either of the other organisms.

Since it was observed that the organism grew better in the absence of $CaCO_3$, additional determinations for nitrogen fixation were made by the use of liquid media having the same composition as those used previously, with the exception that a small amount of $CaCl_2$ was used in place of the $CaCO_3$. Four sources of carbon were used: sucrose, glucose, mannitol, and dextrin. As shown in table 3, unusually large amounts of nitrogen were fixed during incubation at 30°C. for the long period of 59 days. The results verify the previous observations that the organism fails to develop upon dextrin. It is apparent that the organism will grow and fix nitrogen upon mannitol, but development was not nearly so abundant as that upon sucrose and glucose.

TABLE 3

*Nitrogen fixation in liquid media**

ORGANIC MATERIAL	INOCULUM	TOTAL NITROGEN	NITROGEN FIXED
		mgm.	*mgm.*
Sucrose	Uninoculated	1.2	....
Sucrose	Culture 2	19.9	18.7
Sucrose	Culture 3	20.6	19.4
Glucose	Uninoculated	1.3	....
Glucose	Culture 2	15.9	14.6
Glucose	Culture 3	14.8	13.5
Mannitol	Uninoculated	1.2	....
Mannitol	Culture 2	11.1	9.9
Dextrin	Uninoculated	1.7	....
Dextrin	Culture 2	1.7	0
Dextrin	Culture 3	2.0	0.3

* 100-cc. portions of media containing 1.5 gm. of the organic material. Incubation at 30°C. for 59 days.

DISCUSSION

There can be little doubt that the organism in question is a species of *Azotobacter*. The relatively large oval cells are Gram-negative, are motile in physiologically young cultures, develop well and fix atmospheric nitrogen in media free from combined nitrogen. The organism fails to grow in peptone medium and produces abnormal cells when certain nitrogenous materials are added to carbohydrate media. Its distinguishing characteristics will be evident from a brief comparison with the other species of *Azotobacter*.

The organism is considerably smaller than *Az. agilis*, has much more elongated cells, and does not show the general active motility so characteristic of *Az. agilis* (1, 9, 21). It produces no green pigment but cannot be distinguished from *Az. agilis* by this difference alone, since Kluyver and Van Reenen obtained a strain of *Az. agilis* which formed no pigment (9). Other cultures obtained by Kluyver and Van den Bout (8) produced a soluble pigment like the culture originally described by Beijerinck (1). Winogradsky

isolated cultures with a golden yellow pigment (21). According to Kluyver and Van Reenen and Winogradsky, *Az. agilis* is a typical water organism, and there is little likelihood that it would be found in soils.

Az. vinelandii is readily distinguished from the new organism by pigment production and the formation of a pellicle on liquid media. *Az. vinelandii* produces a soluble green pigment much the same as that of *Az. agilis*, whereas the new organism produced no pigment on the media used during incubation for many days. After 2 or 3 weeks, agar slant cultures acquired a light buff to reddish brown appearance. The liquid media remained colorless. *Az. vinelandii* produces a membrane on liquid media; the new organism, on the other hand, produces turbidity of the liquid media and some sediment and thin deposit on the walls of the flasks, but no pellicle has ever been observed. Although the originally described culture of *Az. vinelandii* was isolated from soil (12), Winogradsky found that the organism could be much more readily recovered from water than from soil (21).

Az. chroococcum also forms a pellicle on liquid media, and the typical cultures can be further distinguished from the new organism by the formation of a deep brown to black pigment. Strains with little or no color are frequently encountered, but possibly these might be induced to produce the pigment if adequate copper was supplied. Mulder (18) found that *Az. chroococcum* produced no pigment in media lacking copper but that the brown-black color appeared when 5γ of copper were added to each plate of agar. The influence of copper on pigmentation of the new organism was tested by using agar slants containing glucose and sucrose. From 0.1 to 10γ of copper (as $CuSO_4$) were added per 10 cc. of agar in the tubes. In no case was there evidence of any pigmentation during the incubation period of 14 days.

Az. beijerinckii is nonmotile and produces chains of cells, whereas the new organism is motile and has shown no evidence of chain formation.

Winogradsky reported that *Az. agilis* differs from the other species of *Azotobacter* in that it never forms cysts and that there is little evidence of change in the appearance of the cells during prolonged incubation. The new organism is much the same. As the culture ages, scarcely any difference can be noted in the appearance of the cells except that some cells become a bit more round. The only condition under which unusually shaped cells have been seen is where nitrogenous materials were incorporated in the media. There was no evidence of cysts in any of the cultures, but there may still be some question as to whether or not the organism produces cysts, since Winogradsky observed that cysts were formed less readily when the cultures of *Azotobacter* were grown on carbohydrate media than upon ethanol or butanol. Neither of these two materials was used in the present study. *Az. agilis* makes scant development upon mannitol (8, 9, 21). When isolated, the new organism likewise failed to make appreciable growth upon mannitol but, after being kept in culture for many months, it grew in mannitol solution, although development was slow and not nearly so abundant as upon sucrose and glucose. It is interesting that the organism has many characteristics similar to those of *Az. agilis*, which

is essentially a water bacterium. In this connection, it should be recalled that the new organism was isolated from rice soils which are flooded for a considerable time each year.

Although the cells of the new organism differ relatively little in size or shape from those of some other *Azotobacter*, it is apparent from this short comparison that various cultural and morphological characteristics clearly distinguish this organism from any of the previously described species. The most evident and distinguishing characteristics of the new organism, however, are the appearance of the cells and the slime formation. The rod-shaped cells with a large globule of fatty material at each end have a quite different appearance from the cells of any of the other *Azotobacter*. Although fatty substances and volutin occur in other species, they are distributed irregularly in the cells, which consequently have a granular appearance. The cells growing upon agar media are imbedded in a profusion of slimy material which is much more tenacious and elastic than that produced by other species of *Azotobacter*.

Thus it seems that the characteristics of the organism are sufficiently different from those of the other species of *Azotobacter* to justify the conclusion that it is a new species. The name *Azotobacter indicum* is therefore proposed for this organism isolated from soils of India.

SUMMARY

Cultures of nitroten-fixing bacteria were isolated from soils of India which were acid in reaction (pH 4.9–5.2). They all had similar characteristics which differed in many respects from those of the previously described species of *Azotobacter*. It is concluded that the organism is a new one, and the name, *Azotobacter indicum nov. spec.*, has been proposed.

The organism develops somewhat more slowly than other species of *Azotobacter*. It grows well in nitrogen-free media which are neutral or slightly alkaline in reaction, using sucrose and glucose as sources of energy. It grew very slowly upon mannitol, but considerable amounts of nitrogen were fixed in a solution medium containing this material during prolonged incubation. Dextrin was not utilized. Growth was much restricted in media containing $CaCO_3$.

The cells, which measure 0.5–1.2 by 1.7–2.7μ, are Gram-negative and motile. They differ principally from the cells of other species of *Azotobacter* in that there is a large fat globule at each end of the rod-shaped cells. The cells changed very slightly in morphology during prolonged incubation in nitrogen-free carbohydrate media but became abnormal in media containing certain nitrogenous materials. No cysts or spores were detected.

Growth on agar was at first colorless but became buff to reddish brown during incubation for 3 weeks or longer. Large amounts of slime, characterized by great tenacity and elasticity, are produced. No pigment was produced in liquid media and no pellicle was formed. The liquid became turbid, and some sediment and deposit formed on the walls of the flask.

Tests of the nitrogen-fixing capacity of the organism, in both solid and liquid

media, gave results similar to those obtained with other species of *Azotobacter*. As much as 13 mgm. of nitrogen were fixed per gram of sucrose supplied in a favorable liquid medium. Under similar conditions nearly 10 mgm. of nitrogen were fixed from glucose and about 6.5 mgm. from mannitol.

REFERENCES

(1) BEIJERINCK, M. W. 1901 Ueber oligonitrophile Mikroben. *Centbl. Bakt.* (II) 7: 561–582. [See also Verz. Geschr. van M. W. Beijerinck, 4: 105–124, Delft, 1921.]

(2) DE, P. K. 1936 The nitrogen supply of rice. 1. Fixation of nitrogen in the rice soils under waterlogged conditions. *Indian Jour. Agr. Sci.* 6: 1237–1245.

(3) DE, P. K. 1937 A note on the nitrogen supply of rice soils. *Proc. Natl. Inst. Sci. India* 3: 191–195.

(4) DIETRICH, A., AND LIEBERMEISTER, G. 1902 Sauerstoffübertragende Körnchen in Milzbrandbacillen. *Centbl. Bakt.* (I, Orig.) 32: 858–865.

(5) DOOREN DE JONG, L. E. DEN 1937, 1938 De gedragingen van Azotobacter onder abnormale voedingscondities en radiumbestraling. *Antonie van Leeuwenhoek* 4: 203–211 (1937). Das Verhalten von Azotobacter chroococcum unter abnormen Lebensbedingungen. *Arch. Microbiol.* 9: 223–252 (1938).

(6) EISENBERG, P. 1909 Ueber Fetteinschlüsse bei Bakterien. *Centbl. Bakt.* (I, Orig.) 48: 257–274.

(7) JONES, D. H. 1920 Further studies on the growth cycle of Azotobacter. *Jour. Bact.* 5: 325–341.

(8) KLUYVER, A. J., AND BOUT, M. T. VAN DEN 1936 Notiz über Azotobacter agilis Beijerinck. *Arch. Mikrobiol.* 7: 261–263.

(9) KLUYVER A. J., and REENEN, W. J. VAN 1933 Über Azotobacter agilis Beijerinck. *Arch. Mikrobiol.* 4: 280–300.

(10) LEWIS, I. M. 1937 Cell inclusions and the life cycle of Azotobacter. *Jour. Bact.* 34: 191–205.

(11) LIPMAN, C. B., AND BURGESS, P. S. 1915 Studies on nitrogen fixation and Azotobacter forms in soils of foreign countries. *Centbl. Bakt.* (II) 44: 481–511.

(12) LIPMAN, J. G. 1903 Experiments on the transformation and fixation of nitrogen by bacteria. *N. J. Agr. Exp. Sta. Ann. Rpt.* 24: 217–285.

(13) LIPMAN, J. G. 1904 Soil bacteriological studies. Further contributions to the physiology and morphology of members of the Azotobacter group. *N. J. Agr. Exp. Sta. Ann. Rpt.* 25: 237–289.

(14) LÖHNIS, F. 1921 Studies upon the life cycles of the bacteria. Part 1. Review of the literature, 1838–1918. *Mem. Natl. Acad. Sci.* 16 (second mem.): 1–335.

(15) LÖHNIS, F., AND HANZAWA, J. 1915 Die Stellung von Azotobacter im System. *Centbl. Bakt.* (II) 42: 1–8.

(16) LÖHNIS, F., AND WESTERMANN, T. 1909 Ueber stickstoffixierende Bakterien. IV. *Centbl. Bakt.* (II) 22: 234–254.

(17) MEYER, A. 1912 Die Zelle der Bakterien. Gustav Fischer, Jena.

(18) MULDER, E. G. 1938 Over de beteekenis van koper voor de groei van planten en micro-organismen. Dissertation, Wageningen.

(19) STAPP, C. 1924 Über die Reserveinhaltstoffe und den Schleim von Azotobacter chroococcum. *Centbl. Bakt.* (II) 61: 276–292.

(20) WINOGRADSKY, S. 1932 Études sur la microbiologie du sol. Analyse microbiologique du sol, principes d'une nouvelle méthode. *Ann. Inst. Pasteur* 48: 89–134.

(21) WINOGRADSKY, S. 1938 Études sur la microbiologie du sol et des eaux. Sur la morphologie et l'oecologie des Azotobacter. *Ann. Inst. Pasteur* 60: 351–400.

PLATES

PLATE 1

FIG. 1. Colony development of *Az. indicum* upon silica gel plate containing sucrose and seeded with granules of soil. Incubated about 3 weeks at 30°C. Upon continued incubation the few colonies continued to develop and spread over one-half of the plate. 0.65 ×.

FIG. 2. Development of *Az. indicum* on streaked plate of nitrogen-free sucrose agar. Incubated 8 days at 30°C. 0.56 ×.

FIG. 3. Colonies of *Az. indicum* on glucose agar plate. Diluted cell material mixed with the agar before pouring of the plate. Round, glistening, raised, surface colonies and lens-shaped subcolonies. Incubated 9 days at 30°C. 2.4 ×.

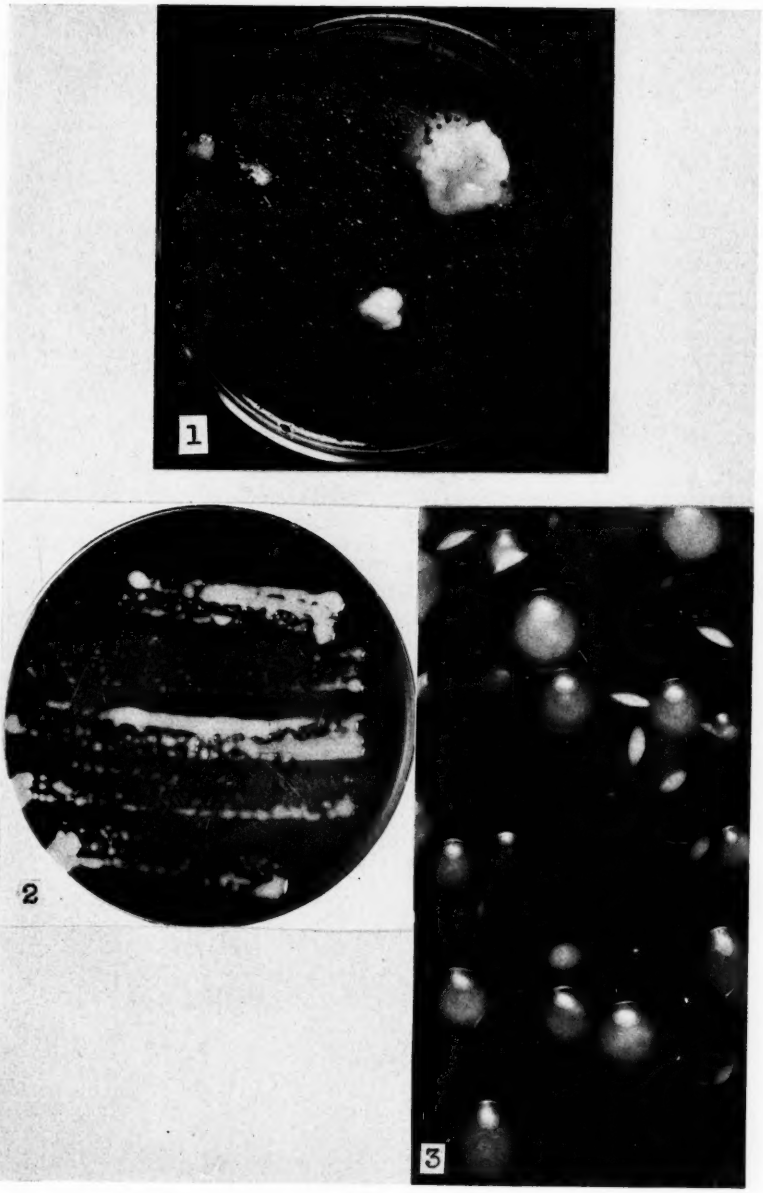
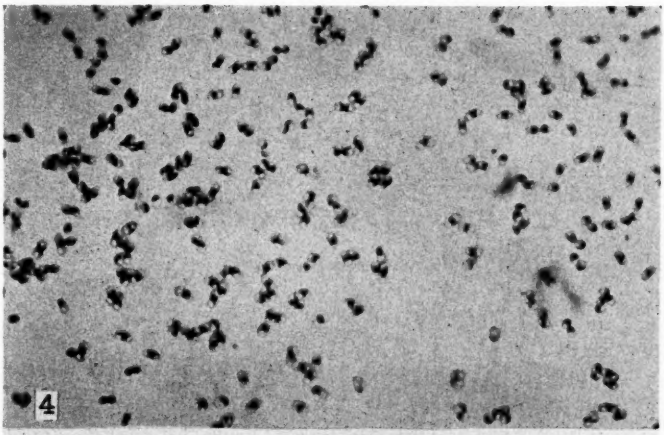
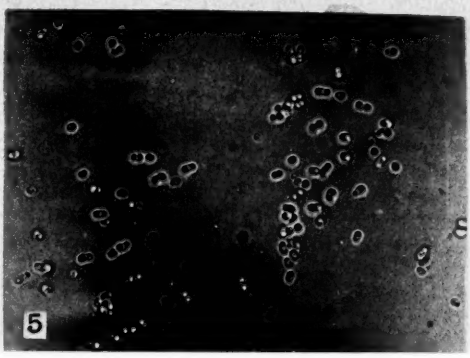

1
2
3

PLATE 2

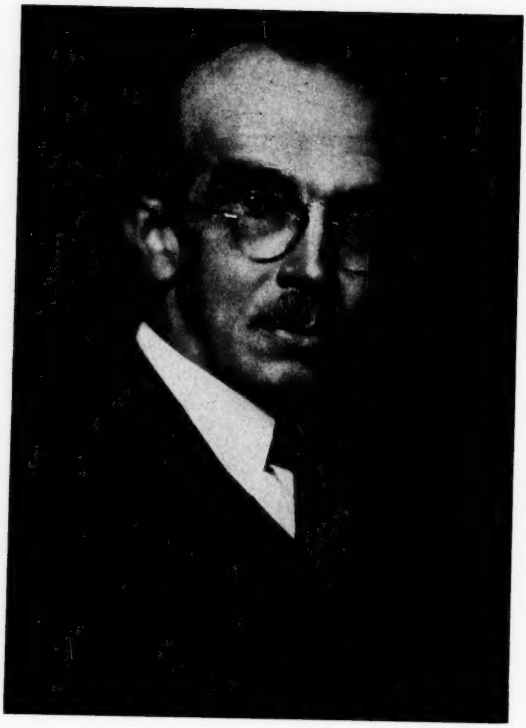
FIG. 4. Cells of *Az. indicum* stained with phenolic rose-bengal. The terminal bodies composed of fatty material remain colorless, only the central portion of the cell being stained. 1200 ×.

FIG. 5. Unstained cells of *Az. indicum*. Cells suspended in warm gelatin (10 per cent) and spread in a thin film beneath a cover slip. The highly refractive terminal globules of fatty material are clearly distinguished from the central region of the cells. Each cell is surrounded by a zone suggesting the presence of material differing from the suspending liquid. 925 ×.





UNIV. OF MICH.



Edmund Cecil Shorey

UNIV. OF MICH.

Edmund Cecil Shorey

1865-1939

Edmund C. Shorey, Fellow of the American Institute of Chemists, Inc., passed away after a considerable period of illness early Monday morning, January 30, at Emergency Hospital, Washington, D. C.

Dr. Shorey was born in Lanark County, Ontario, on March 5, 1865. He pursued both his undergraduate and his graduate work at Queens University, Kingston, Ontario. The B. A. degree was granted in 1886; the M. A., in 1887; and the D. Sc., in 1896. This institution also honored him with a gold medal in chemistry and a silver medal in natural science.

For the year following his graduation he was connected with Queens University as a demonstrator in chemistry. From 1888 to 1891 he was engaged in private analytical work and assaying, the time being divided about equally between Kingston, Ontario, and San Francisco, California.

Dr. Shorey entered the field of industrial chemistry when he became chemist for the Kohala Sugar Company, of Hawaii. This was not only his first major activity, but it was the first time the sugar industry of the Islands employed a chemist to direct production. This experience was supplemented by study at Audubon Park Sugar School, New Orleans, where Dr. Shorey spent three months with the Louisiana Experiment Station investigating cane growing and sugar making. In 1899 he became chemist of the Territory of Hawaii and served in this capacity until 1903. The transition from industrial chemistry to research in a field that later held his major attention was made by Dr. Shorey when he next became chemist of the Hawaii Agricultural Experiment Station. After four years with this institution he was transferred to the Division of Soil Fertility Investigations, Bureau of Soils, U. S. Department of Agriculture.

His long association with the Department was interrupted only by a brief return to commercial research, when from 1918 to 1921, he served the National Aniline and Chemical Company as research chemist and devoted the following year in a similar capacity to the Semet Solvay Corporation. He served the Department chiefly with the Division of Soil Fertility Investigations, where he attained the rank of senior biochemist. He was also in charge of chemical investigations of the Bureau of Soils for three years. Upon retirement, in 1935, he remained as a collaborator in the Division of Soil Fertility Investigations.

Dr. Shorey's chief interest and activity in chemical research was the biochemical aspect of the organic matter of soils as related to plants. This research is covered by numerous papers and bulletins, and the results of this

work gained him international recognition. His intense interest in this field of organic chemistry was maintained until the last, as evidenced by the publication last March of his work upon the isolation and identification of allantoin from several soils. He was actively engaged in similar work up to early last fall.

The fund of chemical knowledge acquired in his varied career was always willingly shared with all who approached him. This reflects the outstanding characteristic of Dr. Shorey as a man, namely, cheerful helpfulness. He possessed to a marked degree a sense of fairness, which was always manifest in dealing with his associates. Although it cannot be said that Dr. Shorey rode any of his varied activities to the point of making them hobbies, he did maintain a keen interest in the reading of detective stories, fishing, photography, and radio construction.

Dr. Shorey was a member of the American Association for the Advancement of Science, the American Chemical Society, the Society of Biological Chemists, and the Washington Academy of Sciences.

OSWALD SCHREINER.

Jacob Goodale Lipman

1874-1939

DR. JACOB G. LIPMAN, Editor-in-Chief of this *Journal,* died of coronary thrombosis on April 19, at the age of 64.

He established *Soil Science* in 1916, as a Rutgers College publication, open to workers in soil science throughout the world. He carried it through the intervening twenty-four years, without interruption. His aim was to develop our knowledge of the science of the soil and to place this science in the front rank of the natural sciences. In this he was singularly successful. To the many who had the privilege of coming in contact with him, his brilliant mind, his clear vision, and his human sympathy have been an inspiration.

In the death of Dr. Lipman, agriculture, in general, and soil science, in particular, have suffered an irreparable loss.

CAPILLARY TENSION AS A MEASURE OF THE PORE SPACE UNOCCUPIED BY WATER IN SOME DENSE ORCHARD SUBSOILS

DAMON BOYNTON

Cornell University

Received for publication November 1, 1938

In connection with studies of seasonal variation of oxygen and carbon dioxide in some dense orchard subsoils, it became desirable to have a continuous approximate measurement of the opportunity for gaseous diffusion in the soil layers under consideration. The pore space (capillary and non-capillary) and the proportion of it unoccupied by water clearly limit the opportunity for diffusion of gases in a given soil layer at any time. This study was undertaken to determine whether or not the tensiometer described by Richards and Neal (6) could be calibrated with sufficient accuracy in terms of the pore space unoccupied by water in these rather heavy subsoil layers.

CALIBRATION OF THE TENSIOMETERS

The calibration was accomplished in a way very similar to that described by Neal, Richards, and Russell (5). A soil column with essentially undisturbed structure was isolated on the side of a ditch 5 feet deep. An iron pipe 6 inches long by 3 inches inside diameter, with one end sharpened on the outside, was eased carefully over the column at the desired depth, so that the pipe was completely filled with soil, the natural structure of which was retained. The exposed ends of the soil column were paraffined. A core of soil a little smaller than the porous clay cup used[1] was cut out of the center of each soil column with a knife, and the cup was pressed into the hole. The soil was allowed to absorb water from the cup for about a week, and then manometer connections were made, and the system was closed. One three-eighth inch hole in the paraffin sealing the surface of the column permitted slow drying. Room temperature varied from about 24° to 27°C. during the calibrations. Each cylinder of soil was weighed daily, and the tension on the mercury column was recorded at the same time. In order to minimize the effects of hysteresis (7), care was taken to carry on the calibrations when the soil was drying. The drying curve for each sample was determined three or more times. At the end of the calibrations the percentage of moisture and the volume weight of the

[1] The porous clay cups were made by the General Ceramics Company according to the specifications of L. A. Richards.

samples were determined, and the pore space unoccupied by water was computed from the formula

$$\left[100 - \left(\frac{\text{volume weight}}{2.65} \times 100\right)\right] - \text{Per cent volume occupied by water} = \text{Per cent pore space unoccupied by water.}$$

Previous determinations by the pycnometer method indicated that the true specific gravity of these subsoil layers was very close to 2.65. The volume weights of the samples ranged from 1.45 to 1.64; there was reasonable agreement between duplicates. The highest volume weights, 1.62 and 1.64, were encountered in the 45–51-inch depth of location 1.

The curves in figures 1 and 2 represent the calibrations of duplicate samples at depths of 1, 2, 3, and 4 feet in two variations of Dunkirk silty clay loam. The profile at location 1 (fig. 1) is characterized by heavy silty clay at 12 and 24 inches, and by dense laminated silt at 48 inches. This is in contrast to a rather uniform light silty clay loam throughout the 4-foot section of location 2 (fig. 2).

The curves cover a range in moisture of less then 4 per cent of dry weight and a range in pore space unoccupied by water of less than 5 per cent of total volume. There are differences in the absolute percentages covered by the curves for the different depths and locations that must be due to differences in texture and structure. There are also some differences between duplicate samples that are explicable only on that basis. But it is interesting and seems significant that the gas space when the manometers registered zero tension varied only from 0.0 to 6.6 per cent of total volume. The two layers that had least gas space at zero tension were, in figure 1, the 2-foot and 4-foot depths. Neither sample of the 4-foot depth had any gas space at zero tension. Previous work has indicated that opportunity for aeration in the fourth-foot depth of location 1 is normally very poor (2). Both samples of the second-foot depth had less than 2.5 per cent gas space at zero tension. The soil at that depth is dense reddish brown silty clay. It is the heaviest zone, texturally, under consideration here. The other samples, at zero tension, varied in gas space only from 3.0 to 6.6 per cent of total volume.

DIRECT DETERMINATION OF THE CAPILLARY TENSION AT THE FIELD CAPACITY

According to the definition of Stephenson and Schuster (8) the pore space occupied by water when a soil is at field capacity is the capillary pore space; and pore space unoccupied by water when the soil moisture is at that level is noncapillary pore space. The work of Baver (1), Stephenson and Schuster (8), and the Russian investigators discussed by Krause (4), among others, has indicated that soils whose noncapillary pore space is much below 8 per cent of total volume may be poorly aerated. If the field capacity lies within the range of the tensiometer, it should be possible to use the tensiometer to determine in a given layer at a given time the proportions of capillary and noncapillary pore

space occupied by gas, and thereby to gain a better understanding of the opportunity for gaseous diffusion. For this reason the capillary tension and field capacity of columns of these soils *in situ* were determined.[2]

Close to each of the sampling locations for the calibration study, a ditch 4 feet deep was dug around a column of soil 4 feet square which had previously been saturated with water. The isolated column was wrapped and covered with three layers of waterproof building paper, and a heavy hay mulch was laid over the ditch and the soil column. Porous clay cups were set at depths of 1, 2, 3, and 4 feet and were connected to mercury manometers according to the method of Richards and Neal (6). Four soil moisture samples were taken every week from each column, and the manometer readings were made at the same time. Moisture and tension in the top 3 feet reached equilibrium

TABLE 1

Capillary tension at field capacity, and apparent noncapillary pore space in the calibrated soil layers

	DEPTH			
	12 inches	24 inches	36 inches	48 inches
Location 1				
Capillary tension × 1.08 (cm. Hg) at field capacity..... *cm.*	2.1	1.6	2.0	16.8
Apparent noncapillary pore space				
Sample 1..... *per cent of volume*	5.5	2.8	7.3	1.2
Sample 2..... *per cent of volume*	3.3	2.0	6.5	1.0
Location 2				
Capillary tension × 1.08 (cm. Hg) at field capacity..... *cm.*	2.8	0.4	2.9	6.7
Apparent noncapillary pore space				
Sample 1..... *per cent of volume*	7.8	6.2	7.2	7.5
Sample 2..... *per cent of volume*	5.4	5.4	6.5	7.0

within 3 weeks. Moisture percentages, constant within experimental error, were reached at the 4-foot depths after 6 weeks. The tensions at the field capacity are given in table 1. The manometer readings at the fourth-foot depths were higher than those at the other depths, notably at location 1. In that case the high tension, 16.8 cm., may have been due to the density of the layer.

Unfortunately the field capacity columns, *in situ*, could not be identical with the calibration samples. But the variations in texture and structure seemed small enough to justify the use of the tensions at field capacity in estimating

[2] It has been found in a study of the seasonal fluctuation of soil moisture that the moisture equivalent seems to be higher than the field capacity of these subsoil layers (3). In all calibration samples but one there was no tension on the mercury column at the percentage of moisture corresponding to the moisture equivalent of the sample.

the apparent noncapillary pore space of the calibration samples. The resulting figures are presented in table 1. Figures 1 and 2 show that in these layers at zero tension part or all of the noncapillary pore space is filled with water, and at maximum tension all of the noncapillary and part of the capillary pore space is occupied by gas.

CONCLUSIONS

On the basis of this study, it is concluded that the tensiometer can be used with caution to estimate the pore space unoccupied by water in these dense subsoils. Since variations occur in soil texture and structure, the calibrations must apply only to apparently uniform layers in restricted areas and even then are subject to the possibility of error. Although the range of pore space covered by the tensiometer is small, it seems possible that the most critical range is covered, insofar as the aeration of these soils is concerned.

REFERENCES

(1) BAVER, L. D. 1933 Soil porosity as an index to structure. *Amer. Soil Survey Assoc. Rpt. Bul.* 14: 83–85.

(2) BOYNTON, D. 1938 Concerning the pore space in two orchard soils of different productivity. *Proc. Amer. Soc. Hort. Sci.* 35 (1937): 315–319.

(3) BOYNTON, D., AND SAVAGE, E. F. 1938 Soils in relation to fruit growing in New York. Part XIII. Seasonal fluctuations of soil moisture in some important New York orchard soil types. N. Y. (Cornell) Agr. Exp. Sta. Bul. 706.

(4) KRAUSE, M. 1931 Russische Forschungen auf dem Gebiete der Bodenstruktur. *Landw. Jahrb.* 73: 603–690.

(5) NEAL, O. R., RICHARDS, L. A., AND RUSSELL, M. B. 1937 Observations on moisture conditions in lysimeters. *Proc. Soil Sci. Soc. Amer.* 2: 35–44.

(6) RICHARDS, L. A., AND NEAL, O. R. 1936 Some field observations with tensiometers. *Proc. Soil Sci. Soc. Amer.* 1: 71–91.

(7) RICHARDS, S. J., AND LAMB, J., JR. 1937 Field measurements of capillary tension. *Jour. Amer. Soc. Agron.* 29: 772–780.

(8) STEPHENSON, R. E., AND SCHUSTER, C. E. 1937 Physical properties of soils that affect plant nutrition. *Soil Sci.* 44: 23–37.

PRESERVATION OF SMALL CORE SOIL SAMPLES

R. W. GERDEL[1]

U. S. Department of Agriculture

Received for publication October 24, 1938

A method for preserving small core soil samples by impregnation with an unpolymerized plastic and subsequent polymerization has given very satisfactory results. This method of preservation permits frequent handling of the sample without disintegration of the structure, and a suitably collected and preserved series of samples permits direct comparison of structural changes throughout the season. The preserved samples retain almost all of the structural features which are observed in the fresh field samples, and, in some soils, these structural differences are greatly emphasized by this process of preservation. Photographs of a few preserved core samples are presented in plate 1.

Neither the technic nor the equipment required for preservation of these core samples is complicated. Short sections of 2-inch galvanized sheet iron rain spouting are used to collect and hold the samples. These sections may be of any desired length, but 2- to 3-inch lengths are most easily handled. Samples are collected either by driving the short section of tubing into the soil or by collecting a large clod and carefully shaving it into a cylinder to fit the section of tubing. If the samples are very high in moisture content, overnight drying in a low temperature oven is advisable.

A small section of wire window screen is placed in the bottom of a beaker, the tube and enclosed soil core placed on this screen, and another piece of screen placed on top of the core. Bakelite XR-7929 is then poured into the beaker until just level with the top of the soil in the tube. The beaker and soil core are then placed in a vacuum desiccator under moderate vacuum for 2 to 4 hours. An ordinary water jet vacuum pump will produce sufficient vacuum for this purpose. The length of time required for complete impregnation will vary with the density of the soil core. Further additions of the unpolymerized plastic are made from time to time, keeping the level in the beaker equal to the height of the soil core. Infiltration of the plastic may be hastened in very wet or very dense samples by placing the desiccator over a hot plate and maintaining a temperature of 70° to 80°C. while the vacuum is applied.

After complete impregnation of the soil core with the plastic, the tube and contents are removed from the beaker, placed upon a small tin pan, and kept in an oven at 80°C. for 48 to 72 hours to ensure complete polymerization. The

[1] Assistant soil technologist, Soil Conservation Service, Zanesville, O.

cemented soil core may then be removed from the tube, and one or more faces ground upon an emory wheel and polished with fine emory paper.

Should a soil core become broken at any time, it may be cemented together with Bakelite BR-0014 and reheated in the oven, leaving no trace of the break.

Since Bakelite XR-7929 is slightly water soluble in the unpolymerized state, it is miscible with the soil solution and apparently does not produce serious structural changes.

PLATE 1

SOIL CORE SAMPLES PRESERVED WITH BAKELITE; ONE FACE GROUND AND POLISHED TO SHOW STRUCTURE (SLIGHTLY REDUCED)

FIG. 1. Core samples from fallow ground showing development of an erosion pavement by late winter and spring rains.

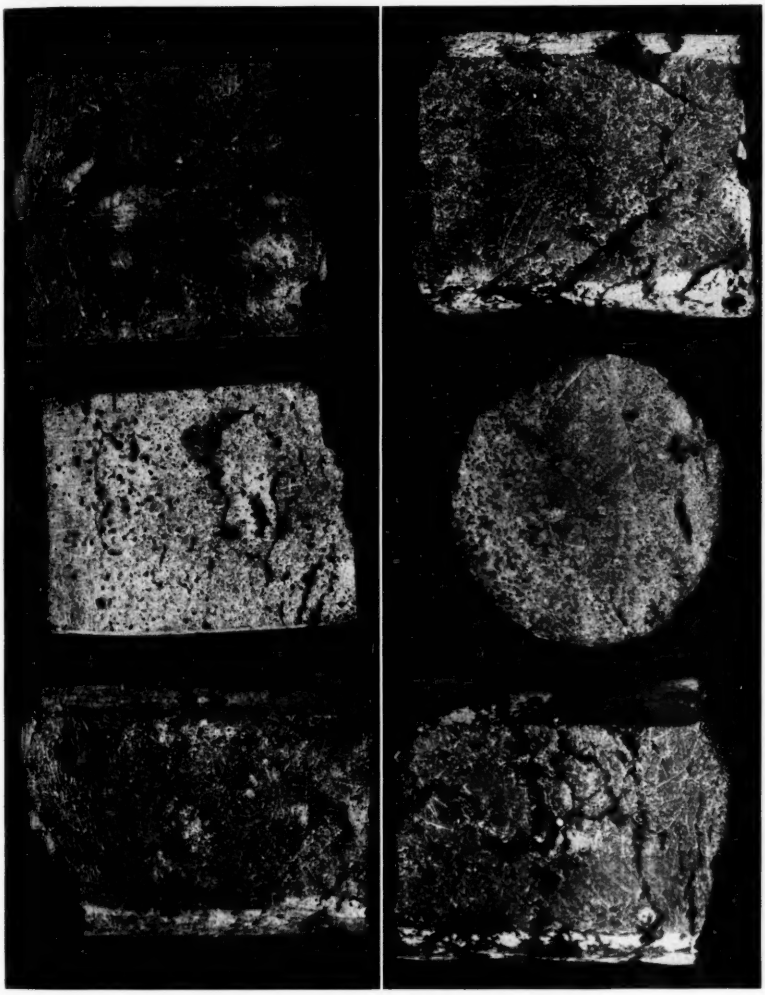
Left—freshly plowed, January, 1938; center—same field, March, 1938; right—same field, May 1, 1938.

FIG. 2. Variations in type of structure.

Left—under heavy sod in old fence row; center—horizontal core showing impervious strata; right—under sod in pasture, field previously cultivated.

FIG. 1

FIG. 2



UNIV. OF MICH.

INFLUENCE OF EXCHANGEABLE CATIONS ON THE AVAILABILITY OF PHOSPHATE IN SOILS[1]

S. RAVIKOVITCH

Agricultural Research Station, Rehovoth, Palestine

Received for publication November 3, 1938

The concentration of phosphoric acid salt solutions introduced into the soil and the kind of exchangeable cations in the complex determine the nature of the phosphate compounds formed. These compounds are the resultants of combinations between phosphate ions of different valences, the exchange complex, and its cations (9). The PO_4 adsorbed might be expected to possess varying degrees of availability, dependent on the character of the combination with the soil complex and its cations. The PO_4 which appears in the soil solution, when the soil comes into contact with acids, alkalies, and salts of concentrations causing only an exchange reaction without destroying the adsorbing complex, should be considered as readily available. The liberation of the adsorbed PO_4 is dependent not only on the anion exchange but also to a great extent upon the cation exchange reaction. The kind of cations present in the soil, when the PO_4 was adsorbed, as well as the changes in the composition of the exchangeable bases after the PO_4 adsorption occurred, influences greatly the degree of PO_4 availability. A study of the availability of the PO_4 which enters into combination with the complex and its bases and of the influence of the various exchangeable cations on the liberation of the adsorbed PO_4 is presented in this paper.

SOILS AND THEIR PREPARATION FOR THE EXPERIMENTS

A sandy soil with an exchange capacity of 10.48 m.e. per 100 gm. was used. The soil was treated with 0.05 *N* HCl in order to remove the exchangeable bases. A part of the soil was allowed to remain entirely unsaturated, and a part was completely saturated with Ca. To "saturate" with PO_4, the H-soil was treated with H_3PO_4 and the Ca-soil with a neutral solution of ammonium phosphate. The concentration of these solutions was 0.08 *N*. At such a low concentration, as was established, the exchangeable Ca is not replaced, and the PO_4 simultaneously enters into union with the exchangeable Ca and the complex according to the formula:

$$\frac{\text{equiv. of exchangeable Ca}}{\text{equiv. of adsorbed } PO_4} = \frac{2}{3}.$$

[1] Part of a thesis presented to the faculty of Rutgers University in partial fulfillment of the requirements for the degree of doctor of philosophy. Other parts of this thesis have been published in Soil Science (9, 10).

The adsorption of the monovalent phosphoric acid is proposed. The trivalent PO_4 ions were adsorbed by the H-soil. At these low concentrations, only a partial anion exchange between the ions of the complex and the phosphoric acid ions occurred (9).

The amount of adsorbed PO_4 in the Ca- and H-soils was adjusted to 0.1885 m.e. for a sample of 7.5 gm. The Mg-, NH_4-, Na-, and K-soils were prepared from the H-soils by complete replacement of the hydrogen by the corresponding cations. After saturation, the soils were brought to air-dry condition and used for the various experiments.

The comparative availability of the PO_4 adsorbed was established by shaking the soil samples (7.5 gm.) with 50 cc. HNO_3 of different concentrations. The soil was left in contact with the solution for 4 days. The supernatant liquid was decanted and analyzed. All calculations were made on the basis of oven-dry matter.

COMPARATIVE DEGREE OF AVAILABILITY OF PO_4 ABSORBED BY Ca-SOILS, BY H-SOILS, AND BY H-SOILS WITH SUBSEQUENT REPLACEMENT OF THE H BY Ca

As a result of treatment of the Ca-soil with acids of very low concentrations (0.001–0.003 *N*), a considerable part of the adsorbed PO_4 (18.5–25.2 per cent) passes into solution (table 1).

The liberation of the PO_4 is not a consequence of an anion exchange with the NO_3 ions, which are virtually unadsorbed or are absorbed in very insignificant amounts, but results from the disturbance of the combination of the exchange complex with the PO_4 and the calcium. This disturbance is brought about by the cation exchange reaction between the Ca of the complex and the H of the acid. The moment the Ca is removed, the PO_4 ions break away from the complex and go into the soil solution. The PO_4 brought into solution by the very weak acids which result only in exchange reaction, may be considered as readily available. With further increase of acid concentration (0.005–0.010 *N*), a very slight breakdown of the complex occurs. The slight decrease in PO_4, at these concentrations, may be explained as due to the readsorption by the partly unsaturated complex at lower pH, as well as to the combination with the aluminum appearing in solution (3). The increase in the amount of the water-soluble PO_4 at still higher concentrations of acid (0.0133–0.0333 *N*), is associated with a marked destruction of the complex.

In another series of experiments (table 2), the stability of combination between the adsorbed PO_4 and the unsaturated complex was investigated. This combination is considerably more stable than that of Ca-soils. The amount of PO_4 liberated from the Ca-soils is markedly higher than that from the H-soils treated with acid solutions of identical concentrations. At concentrations of 0.001–0.003 *N* HNO_3, the PO_4 liberated from the H-soils amounts to only 3.4–4.7 per cent. With a further increase of HNO_3 concentration (0.005–0.010 *N*), the amount of PO_4 is also lower in the H-soil extracts. The liberation of PO_4 from the H-soils at these concentrations is associated with the partial

destruction of the complex, as can be seen from the appearance of aluminum and iron in the solution, whereas in the Ca-soils the PO_4 is liberated chiefly as a result of interference of the established equilibrium between the soil complex and the adsorbed calcium and PO_4. It is only with the higher concentrations (>0.010 *N*) that the amount of PO_4 in the solution is nearly equal in both

TABLE 1

Availability of PO_4 adsorbed by Ca-soils

EXPERIMENT NUMBER	NORMALITY OF HNO_3 SOLUTION	PO_4 IN 50 CC. OF THE EXTRACT*	PERCENTAGE OF PO_4 LIBERATED	Al_2O_3 IN 50 CC. OF THE EXTRACT	Fe_2O_3 IN 50 CC. OF THE EXTRACT	pH
		m.e.		*m.e.*	*m.e.*	
1	0.001	0.0349	18.5	None	None	
2	0.0015	0.0379	20.1	None	None	
3	0.003	0.0476	25.2	None	None	
4	0.005	0.0549	29.1	Traces	None	6.9
5	0.0066	0.0477	25.3	Traces	Traces	6.7
6	0.008	0.0494	26.2	Traces	Traces	6.3
7	0.010	0.0549	29.1	Traces	Traces	5.6
8	0.0133	0.0621	32.9	0.088	0.009	3.5
9	0.020	0.0705	37.4	0.191	0.017	<2.8
10	0.0333	0.0845	44.8	0.442	0.019	<2.8

* The PO_4 adsorbed by the 7.5-gm. soil sample is 0.1885 m.e.

TABLE 2

Availability of PO_4 adsorbed by H-soils

EXPERIMENT NUMBER	NORMALITY OF HNO_3 SOLUTION	PO_4 IN 50 CC. OF THE EXTRACT*	PERCENTAGE OF PO_4 LIBERATED	Al_2O_3 IN 50 CC. OF THE EXTRACT	Fe_2O_3 IN 50 CC. OF THE EXTRACT	pH
		m.e.		*m.e.*	*m.e.*	
1	0.001	0.0064	3.4	Traces	None	
2	0.0015	0.0059	3.1	Traces	None	
3	0.003	0.0089	4.7	Traces	None	
4	0.005	0.0226	12.0	0.081	0.008	3.5
5	0.0066	0.0323	17.1	0.162	0.009	2.9
6	0.008	0.0352	18.7	0.176	0.010	<2.8
7	0.010	0.0447	23.7	0.264	0.012	<2.8
8	0.0133	0.0579	30.7	0.324	0.012	<2.8
9	0.020	0.0748	39.7	0.529	0.015	<2.8
10	0.0333	0.0845	44.8	0.700	0.017	<2.8

* The PO_4 adsorbed by the 7.5-gm. soil sample is 0.1885 m.e.

series. At these concentrations of HNO_3, the Ca-complex is also destroyed, but the amounts of aluminum and iron in the solution are considerably lower as compared with the H-soils. The liberation of the trivalent PO_4 ions from the H-complex seems to be as difficult as their adsorption (9).

Another series of experiments was carried out with the same PO_4-saturated

H-soil, with the exception that the hydrogen was completely replaced by Ca after the PO_4 was adsorbed (table 3). This corresponds to liming of acid soils previously fertilized with phosphates. The introduction of Ca ions preserves the complex from destruction when it comes in contact with acids. The Ca ions block the approach of the acid to the nucleus bearing the PO_4 ions and partly neutralize the acid introduced. The quantities of PO_4 which appear in the solution at all concentrations of HNO_3 are markedly smaller than the quantities which appear in the extracts of H-soils (table 2).

As follows from the data presented, the PO_4 adsorbed by the Ca-soil is the most readily available. The combination of the soil complex with the PO_4 and Ca proved to be very unstable also toward water. Of the total adsorbed PO_4, 15 per cent appears in the solution when the 7.5-gm. soil sample is shaken

TABLE 3

Availability of PO_4 adsorbed by H-soils after replacement of H by Ca

EXPERIMENT NUMBER	NORMALITY OF HNO_3 SOLUTION	PO_4 IN 50 CC. OF THE EXTRACT*	PERCENTAGE OF PO_4 LIBERATED	Al_2O_3 IN 50 CC. OF THE EXTRACT	Fe_2O_3 IN 50 CC. OF THE EXTRACT	pH
		m.e.		*m.e.*	*m.e.*	
1	0.001	0.0075	4.0	None	None	
2	0.0015	0.0056	3.0	None	None	
3	0.003	0.0042	2.2	None	None	
4	0.005	0.0070	3.7	Traces	None	6.7
5	0.0066	0.0074	3.9	Traces	Traces	6.5
6	0.008	0.0097	5.1	Traces	Traces	6.1
7	0.010	0.0135	7.2	Traces	Traces	5.6
8	0.0133	0.0237	12.6	0.081	0.006	3.5
9	0.020	0.0456	24.2	0.216	0.008	<2.8
10	0.0333	0.0777	41.2	0.470	0.013	<2.8

* The PO_4 adsorbed by the 7.5-gm. soil sample is 0.1885 m.e.

with 50 cc. of distilled water. This combination, under field conditions, can be obtained when a soil containing a relatively large proportion of exchangeable Ca is brought in contact with phosphates at low concentrations. This may be considered in the case of irrigated cultures by supplying the phosphates to the soil in dissolved state in a dilute concentration. An easily available and mobile form of PO_4 in the soil can be thus obtained.

Influence of exchangeable Na, K, NH_4, H, Mg, and Ca on the liberation and adsorption of PO_4

The entrance of any one cation into the complex changes the physico-chemical state and reaction of the soil. These changes, as they affect the nature and rate of PO_4 adsorption, also affect the degree of liberation of the PO_4 already adsorbed before the base exchange occurred (10). To ascertain the influence of the different exchangeable bases upon the liberation of the

PO_4 adsorbed, two series of experiments were carried out on two soils. The hydrogen ions of sandy and clay H-soil samples saturated with PO_4 ions were

TABLE 4

Liberation of adsorbed PO_4 as influenced by various exchangeable cations (water extract)*

EXPERIMENT NUMBER	KIND OF CATION INTRODUCED IN THE COMPLEX	PO_4 FOUND IN 50 CC. OF THE EXTRACT	PERCENTAGE OF PO_4 LIBERATED	pH
		m.e.		
Sandy soil				
1	Ca	0.0091	3.1	7.05
2	Mg	0.0180	6.1	7.05
3	H	0.0190	6.4	6.65
4	NH_4	0.0391	13.2	7.10
5	K	0.0423	14.2	7.10
6	Na	0.0913	30.7	7.30
Clay soil				
1	Ca	0.0401	10.4	7.05
2	Mg	0.0464	12.1	7.05
3	H	0.0581	15.1	6.10
4	NH_4	0.0655	17.0	7.10
5	K	0.0718	18.7	7.15
6	Na	0.1690	43.9	7.20

* The 7.5 gm. of sandy soil contains 0.297 m.e. of adsorbed PO_4; the 3.0 gm. of clay soil contains 0.385 m.e. of adsorbed PO_4.

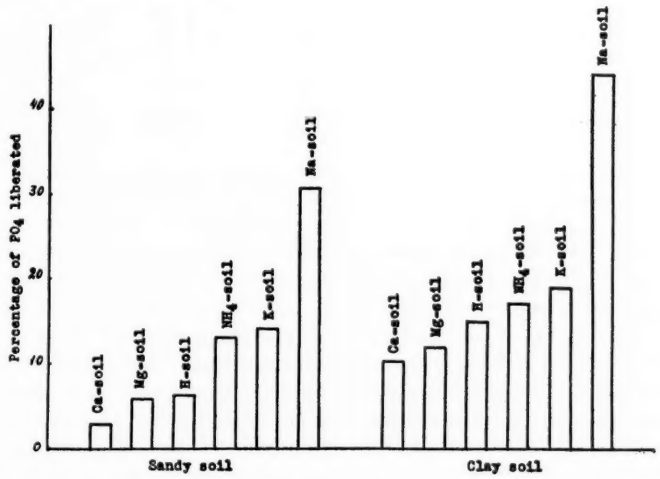


FIG. 1. LIBERATION OF ADSORBED PO_4 AS INFLUENCED BY VARIOUS EXCHANGEABLE CATIONS

replaced by each of the following cations: Ca, Mg, NH_4, K, and Na. The amount of adsorbed PO_4 for 7.5 gm. of sandy soil was 0.297 m.e., and for 3.0

gm. of clay soil 0.385 m.e. For these experiments, only PO_4 which appeared in water extracts was considered. The liberation of PO_4, as seen from the results presented in table 4 and figure 1, was greatest with Na-soils and decreased in the direction to Ca-soils according to the following scheme: $Na > K > NH_4 > H > Mg > Ca$.

The various degrees of PO_4 liberation are mainly in accordance with the changes in the degree of dispersion, swelling, charge, pH, and dissociation of the adsorbed ions, produced in the complex by the different exchangeable cations.

TABLE 5

*Adsorption of PO_4 by soils with various exchangeable cations, from an ammonia phosphate solution**

EXPERIMENT NUMBER	KIND OF CATION IN THE COMPLEX	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY THE SOIL SAMPLE	pH
		m.e.	*m.e.*	
Sandy soil				
1	Ca	6.00	1.174	7.05
2	Mg	6.00	0.968	7.05
3	H	6.00	0.691	6.45
4	NH_4	6.00	0.368	7.10
5	K	6.00	0.314	7.15
6	Na	6.00	0.206	7.15
Clay soil				
1	Ca	6.00	2.093	7.0
2	Mg	6.00	1.311	....
3	H	6.00	1.203	5.8
4	NH_4	6.00	0.503	>7.0
5	K	6.00	0.503	>7.0
6	Na	6.00	0.234	>7.0

* The exchange capacity of the 7.5-gm. sandy soil sample is 0.786 m.e., and that of the 3.0-gm. clay sample, 1.548 m.e. The ratio of PO_4 to NH_4 in the solution equals 1.0 : 0.5 m.e.

To establish the order in which the same cations are arranged according to their effectiveness in adsorption of PO_4, the following series of experiments was carried out. Ca-, Mg-, H-, Na-, K-, and NH_4-soil samples were shaken with 50 cc. of 0.12 *N* neutral ammonium phosphate solution. As far as the fixation of PO_4 is concerned, Ca and Mg are the most effective and Na is the least effective of the exchangeable cations investigated (table 5, fig. 2). The cations which support a higher degree of PO_4 liberation lead to a lower degree of PO_4 retention, and *vice versa*. The effectiveness of the various exchangeable cations in fixing the PO_4 can be indicated by the scheme: $Ca > Mg > H > NH_4 > K > Na$.

The various exchangable cations determine the valency of the phosphate ions adsorbed and thus influence the quantity of adsorption. It was suggested that for Ca-soils at low concentrations which do not involve a replacement of

the exchangeable calcium, the adsorbed ion was the monovalent and for the H-soils the trivalent (9). In these experiments the adsorption of the monovalent ions may be also suggested for the Mg-complex and the trivalent PO_4 ion for the Na-, K-, and NH_4-saturated complexes. The differences in adsorption by soils bearing various monovalent cations, in spite of the proposed adsorption of the ion of the same valency, are governed here too, as in the case of liberation, by the differences produced in the physicochemical state of the complex by the various cations. The same is true for the group of soils bearing the divalent cations.

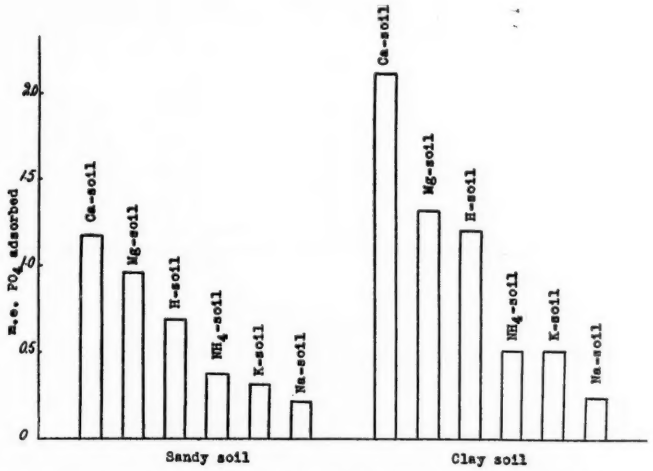


FIG. 2. ADSORPTION OF PO_4 BY SOILS WITH VARIOUS EXCHANGEABLE CATIONS

Availability of tricalcium phosphate as influenced by exchangeable hydrogen

In the fertilization of soils with soluble PO_4 salts, the formation of localized highly concentrated salt solutions leads to cation exchange. The reaction of cation exchange will continuously decrease in all directions with increasing remoteness from the diffusion center of the dissolved salt crystals. In the close vicinity of the dissolving crystal, the PO_4 will connect with the replaced Ca or Mg in the form of dibasic or tribasic phosphate salts. These formed salts will, in time, interact with the H of the complex[2] and will be partly subjected

[2] As a result of fertilization of soils with ammonium or potassium phosphate, the ammonium and potassium ions introduced into the complex will, in time, be partly leached out and partly adsorbed by the plants, leaving H behind in the complex. Joffe and McLean (4) have shown that the exchangeable NH_4 and K are utilized by plants; Magistad (6) and Ravikovitch (8) found that the exchangeable potassium is hydrolyzed and removed from the complex. The introduction of superphosphate into the soil leads also to the increase in the amount of H ions in the complex.

to decomposition. As Cook (1) and Kossovitch (5) have shown, the unsaturated soils increase the availability of rock phosphate.

Table 6 presents the results of experiments on the interaction between tricalcium phosphate and a completely unsaturated mineral exchange complex. Samples of 1.50 gm. of clay H-soil with an exchange capacity of 0.774 m.e.

TABLE 6

Decomposition of tricalcium phosphate by an unsaturated mineral soil exchange complex (water extract)

EXPERIMENT NUMBER	TIME OF CONTACT BETWEEN H-COMPLEX AND $Ca_3(PO_4)_2$*	PO_4 FOUND IN 50 CC. OF THE EXTRACT	PERCENTAGE OF WATER-SOLUBLE PO_4	pH
	days	*m.e.*		
1	2	0.317	41.0	
2	3	0.371	47.9	6.4
3	5	0.303	39.1	6.4
4	7	0.290	37.5	6.3
5	12	0.290	37.5	6.3
6	20	0.283	36.6	6.3

* The 1.5-gm. soil sample contains 0.774 m.e. of exchangeable H, the amount of $Ca_3(PO_4)_2$ added equals 0.774 m.e.

TABLE 7

Decomposition of tricalcium phosphate by an unsaturated organic exchange complex (water extract)

EXPERIMENT NUMBER	TIME OF CONTACT BETWEEN H-COMPLEX AND $Ca_3(PO_4)_2$	$Ca_3(PO_4)_2$ ADDED TO H-PEAT SAMPLE*	PO_4 FOUND IN 50 CC. OF THE EXTRACT	PERCENTAGE OF WATER-SOLUBLE PO_4	pH
	days	*m.e.*	*m.e.*		
1	1	0.662	0.593	89.6	
2	2	0.662	0.613	92.6	
3	3	0.662	0.627	94.7	
4	5	0.662	0.627	94.7	
5	6	0.662	0.627	94.7	
6	2	0.993	0.930	93.7	5.9
7	3	0.993	0.984	99.1	5.9
8	5	0.993	0.984	99.1	5.9

* The 0.50-gm. peat sample contains 0.662 m.e. of exchangeable H.

were brought into contact, in a moist condition, with 0.774 m.e. tricalcium phosphate. After different time intervals, water was added to make 50 cc. of solution. The mixtures were shaken, and water-soluble PO_4 was determined.

Three days of contact between soil and salt gave the maximum of water-soluble PO_4—0.371 m.e. This quantity is actually lower than that which appeared in solution as a result of the tricalcium phosphate decomposition.

A part of the soluble PO_4 is readsorbed by the soil. As is seen, on extended contact between soil and solution, the amount of water-soluble PO_4 decreases.

Another series of experiments with an organic H-complex (lowmoor H-peat) was carried out in order to establish the degree of activity of the exchangeable hydrogen of the organic complex in the decomposition of tricalcium phosphate. The composting of acid peat with rock phosphate or the direct application of rock phosphate to the peat soils is known to be carried out in order to transform the raw phosphate into a more available form (7). In these experiments (table 7), the quantitative relationship between the H of the peat and the Ca of the $Ca_3(PO_4)_2$ in the exchange reaction, which leads to the decomposition of this salt, is considered.

Half-gram samples of H-peat with an exchange capacity 0.662 m.e. were moistened and brought in contact with 0.662 m.e. of $Ca_3(PO_4)_2$. After 1 day of contact, more than 89 per cent of this salt was transformed to a soluble form, and after 3 days of contact this had reached approximately 95 per cent. The water-soluble PO_4 which appeared in the solution may be regarded as free phosphoric acid or as a mixture of this acid with $Ca(H_2PO_4)_2$. In the latter case the complex is left partly unsaturated. A mixture of H-peat with $Ca_3(PO_4)_2$ in the proportion of 2 m.e. of exchangeable hydrogen to 3 m.e. of introduced salt brings about a complete decomposition of the latter (99.1 per cent) with the formation of a Ca-peat and water-soluble $Ca(H_2PO_4)_2$ salt. All the PO_4 remains in solution, since the organic complex, as has been established (2, 9), has practically no PO_4-adsorption capacity.

SUMMARY

The degree of availability of the adsorbed PO_4 by Ca-soils, H-soils, and soils in which the H was replaced by Ca, after being saturated with PO_4, was determined.

The influence of the exchangeable Ca, Mg, NH_4, K, Na, and H on the degree of adsorption and liberation of PO_4 was studied, as was the role of the exchangeable H in the decomposition of $Ca_3(PO_4)_2$.

The greatest availability was established for the PO_4 adsorbed by the Ca-soil. The combination of the soil complex with the PO_4 and exchangeable calcium was proved to be very unstable, breaking down under the action of very weak acids.

The availability of the PO_4 adsorbed by the H-complex is low, and its liberation is associated with the partial destruction of the complex.

The introduction of Ca into the H-complex containing PO_4 in an adsorbed state increases the stability of the complex and decreases the degree of PO_4 liberation.

The effectiveness of the various exchangeable cations in liberation of the adsorbed PO_4 can be indicated by the following scheme: $Na > K > NH_4 > H > Mg > Ca$. These cations, according to their effectiveness in PO_4 ad-

sorption, are arranged in a reverse order as can be seen from this scheme: $Ca > Mg > H > NH_4 > K > Na$.

The contact of mineral and organic H-complexes with $Ca_3(PO_4)_2$ leads to the solution of the salt. Whereas the dissolved phosphate is in part adsorbed by the mineral complex, it is not retained in any appreciable quantity by the organic complex.

REFERENCES

(1) Cook, R. L. 1935 Divergent influence of degree of base saturation of soils on the availability of native, soluble and rock phosphate. *Jour. Amer. Soc. Agron.* 27: 297–311.

(2) Doughty, J. L. 1935 Phosphate fixation in soil particularly as influenced by organic matter. *Soil Sci.* 40: 191–202.

(3) Gaarder, T. 1930 Die Bindung der Phosphorsaure im Erdboden. Vestlandets Forstl. Forsøkssta. Meddel. 14.

(4) Joffe, J. S., and McLean, H. C. 1927 Availability of replaceable cations. *Proc. and Papers First Internatl. Cong. Soil Sci.* 2: 256.

(5) Kossovitch, P. 1909 Pflanze Phosphorit und Boden nach Versuchen des Landwirtschaftlichen Chemischen Laboratoriums, St. Petersburg. *Zhur. Opytn. Agron.* (Russian) 10: 839–842.

(6) Magistad, O. C. 1928 The hydrolysis of sodium and potassium zeolites with particular reference to potassium in soil solutions. Ariz. Agr. Exp. Sta. Tech. Bul. 22.

(7) Prianishnikov, D. N. 1922 Fertilizers [Uchenie ob Udobrenii], govt. ed. Moscow.

(8) Ravikovitch, S. 1930 Exchangeable cations and lime requirement in differently fertilized soil. *Soil Sci.* 30: 79–95.

(9) Ravikovitch, S. 1934 Anion exchange: I. Adsorption of the phosphoric acid ions by soils. *Soil Sci.* 38: 219–239.

(10) Ravikovitch, S. 1934 Anion exchange: II. Liberation of the phosphoric acid adsorbed by soils. *Soil Sci.* 38: 279–290.

EFFECT OF DILUTION ON THE WATER-SOLUBLE AND EXCHANGEABLE BASES OF ALKALI SOILS AND ITS BEARING ON THE SALT TOLERANCE OF PLANTS

W. P. KELLEY

University of California

Received for publication October 24, 1938

In the determination of the replaceable bases of alkali soils, allowance must necessarily be made for the water-soluble bases that are present. As is well known, variations in the ratio of soil to water, used in determining the water-soluble bases, tend to influence the results both quantitatively and qualitatively. This suggests that corresponding changes may be produced on the exchangeable bases of the soil. If so, how important is this effect?

Eaton and Sokoloff (2) concluded that dilution to the extent of 5 parts, or even less, of water to 1 part of soil affects to an important degree the ratio of exchangeable Ca to exchangeable Na. They based their conclusion on the fact that the amounts of water-soluble Na found increased with dilution, whereas the water-soluble Ca tended to decrease with dilution. They reported that with one soil the solution, obtained by a certain displacement procedure, contained 144 m.e. Na per liter, whereas a 1 to 5 water extract of the same soil contained an amount of Na which, if expressed on the basis of the displaced solution, was equivalent to 205.8 m.e. per liter. With another soil the corresponding data were 92.4 and 170.6 m.e. Na, respectively. Eaton and Sokoloff explained their results by assuming that the relative base-exchange activities of Ca and Na ions are influenced by dilution, a theory which agrees in principle with Vanselow's (9) results obtained in Ca-NH_4 equilibrium studies, and that solid phase $CaCO_3$ and $CaSO_4$ in the soil tend to dissolve upon dilution. The Ca ions, thus brought into solution, it was assumed, then replaced absorbed Na and thereby produced an increase in the dissolved Na. Since, as these investigators pointed out, the amount of water-soluble Na found is taken into account in the determination of the exchangeable Na, any error in the determination of the former will be reflected in the latter.

The differences found by Eaton and Sokoloff, when expressed on the basis of the displaced solution, appear large, but, when expressed on the basis of dry soil, they are more modest. For example, 144 m.e. Na, found in the solution displaced from one of the soils referred to above, amounts to only 2.27 m.e. per 100 gm. of the dry soil, and the Na content of a 1 to 5 water extract of this soil was equivalent to 3.69 m.e. per 100 gm. The corresponding figures for the other soil were 1.22 and 2.82 m.e. Na, respectively.

EXPERIMENTAL RESULTS

The writer has investigated two widely different types of alkali soils by means of water displacement, water extraction, and ammonium acetate extraction. One of the soils was taken from an area in the Imperial Valley, California, adjacent to the alkali reclamation experiment which Thomas (8) and Kelley (4, 5, 7) have already discussed. This soil is Holtville clay loam and is extremely high in water-soluble salts. The other soil is Fresno sandy loam taken from an olive orchard on the Kearney Vineyard near Fresno, California. Neither of these soils contains more than traces of water-soluble normal carbonate.

The displacement procedure used was as follows: About 4000 gm. of well-mixed, air-dry soil was spread out in a thin layer and brought to the desired moisture content by the addition of distilled water in the form of fine drops. The soil was then transferred to a stoppered bottle and held for 2 weeks in order to permit uniform distribution of the added water. The amount of water added was just sufficient to increase the moisture content of the soil to approximately that of good tilth. At the end of the 2-week period, the soil was mixed thoroughly and sieved. Samples were taken for moisture determination (at 100°C.), and the remaining soil was immediately packed in a percolator about 5 inches in diameter. The packing was accomplished by putting in a layer of soil about one-half inch deep and tamping it moderately with a large rubber stopper attached to a short rod, repeating this procedure until the required quantity of soil was introduced into the percolator. The amount of soil used provided a soil column approximately 12 inches deep. A head of distilled water about 4 inches deep was placed on the top of the soil column, and displacement was allowed to proceed under the force of gravity without applying pressure.

Clear, displaced solution began to drop from the soil column about 30 minutes after water was placed on its top. This was allowed to continue for almost an hour. Samples of the displaced solution, withdrawn at frequent intervals, showed that the first 90 cc. of the solution collected was practically constant in Cl content. Then the concentration decreased steadily, which was taken to be an indication of dilution with the distilled water placed on top of the soil column. Only the first 75 cc. of the displaced solution was used for analysis. The solution thus obtained from the Imperial Valley soil contained 4475 m.e. Cl per liter; that from the Fresno soil contained 715 m.e. Cl per liter.

Water extracts of the Imperial Valley soil were prepared by adding distilled water to weighed samples of the air-dry soil in amounts calculated to approximate 5-, 10-, 20-, and 30-fold dilutions of the soil at the moisture content used for displacement. After the desired amounts of water were added, the samples were shaken vigorously several times each day for a week then allowed to settle for another week, when it was found that the colloids of the soil were

completely coagulated, leaving a perfectly clear supernatant solution. This was siphoned off for analysis.

A water extract of the Fresno soil was prepared by shaking 200 gm. of dry soil for an hour with 660 cc. distilled water. After filtration through a clay filter, analysis of the filtrate was made. Nitrate must be determined immediately, because it may undergo marked reduction in soil upon standing in contact with water. It was found that the NO_3 content of the displaced solution of this soil also diminished rapidly upon standing. Before the analysis was begun, all the solutions from a given soil, including the displaced solution, were diluted to approximately equal concentration.

TABLE 1

Constituents removed from alkali soils by displacement, water extraction, and ammonium acetate

Results expressed as milliequivalents per 100 gm.

	SOIL-WATER RATIO	HCO_3	Cl	SO_4	NO_3	Ca	Mg	Na
Imperial Valley soil 1861								
Displaced solution	1:0.156	0.04	69.8	0.12	Tr.	22.0	13.5	34.6
Water extract	1:0.80	0.20	70.5	2.62	Tr.	22.3	12.8	37.2
	1:1.30	0.32	68.8	5.75	Tr.	23.6	12.5	37.6
	1:3.20	0.50	67.8	7.34	Tr.	23.8	12.3	39.1
Ammonium acetate extract	.....	.....	.....	.....	.....	.....	.....	39.7
Fresno soil 887								
Displaced solution	1:0.10	0.2	7.1	0.4	*	4.5	2.9	9.4
Water extract†	1:3.3	0.3	6.6	1.5	8.7	3.4	2.7	10.1
Ammonium acetate extract	.....	.....	.....	.....	.....	.....	.....	12.7

* Not reported, because of delay in the time of making the determination, during which nitrate reduction took place.

† Also contained 0.4 m.e. of K.

The analytical results are given in table 1. With the exception of Cl, the results agree, in general, with those reported by Eaton and Sokoloff. They show, in harmony with results previously reported by Kelley and Brown (6) and others, that dilution produced pronounced increases in the total amounts of dissolved HCO_3 and SO_4, which, as Eaton and Sokoloff suggested, were possibly due to the solution of $CaCO_3$ and $CaSO_4$. Another explanation of the SO_4 data, however, is possible, namely, SO_4 may have been adsorbed by soil colloids. If so, dilution would be expected to reduce the absolute amount of adsorption and thus increase the SO_4 in solution.

Dissolved Ca tended to increase with dilution in the Imperial Valley soil, which contains considerable SO_4, and to decrease in the Fresno soil, which contains much less SO_4. On the other hand, Mg decreased slightly upon dilution of both soils, but the amounts found were not greatly beyond the

limits of the error of determination. The Cl found was also practically within the range of the error of determination in each soil. But little support, therefore, was found for the concept of unfree water which Eaton and Sokoloff postulated for their soils. Apparently all of the Cl, and probably NO_3 also, were distributed uniformly throughout the moisture films present at the time of displacement. This is an interesting point, since the calculations were based on the water loss at 100°C.

The Na removed from the Imperial Valley soil by ammonium acetate extraction, which, of course, included both water-soluble and exchangeable Na, was only slightly greater than that removed by water extraction. It is concluded, therefore, that this soil contains but little truly exchangeable Na despite its high concentration of water-soluble Na, a conclusion in harmony with previous results (4, 5, 7, 8). The explanation is found in the high concentration of Ca and Mg present. Unless the concentration of soluble Na exceeds that of Ca plus Mg, Na is unable to replace any important amount of other ions because of the greater replacing activities of the divalent cations. The Fresno soil contains somewhat more exchangeable Na than the Imperial Valley soil, as indicated by the difference between water-soluble and ammonium-acetate-soluble Na. In the Fresno soil the water-soluble Na was almost twice as great as the total soluble Ca and Mg.

DISCUSSION

When an attempt is made to interpret these results in relation to alkali soil reclamation, plant growth, or salt tolerance of plants, it is important to bear in mind that dilution, at least in the upper part of the soil, takes place every time the soil is rained upon or irrigated. In so far as reclamation is concerned, there is, therefore, absolutely no practical significance to the differences found by displacement versus water extraction as regards Na or any other cation. Na, held so loosely as to be removed by mere dilution and drainage, would certainly be leached downward each time irrigation water is applied. Moreover, such Na could hardly produce important adverse physical effects on the soil because of the strong coagulating effect of the soluble salts present.

In all probability differences still greater than those reported in table 1 would be produced with certain types of alkali soil by leaching with water, rather than by making the determination under equilibrium conditions, as is commonly done. In the opinion of the writer, however, the determination of water-soluble Na, made by bringing 1 part of soil to equilibrium with 5 parts of water, gives results which are sufficiently accurate for practical purposes.

It is pertinent to point out in this connection that colloid chemists are well aware that many different kinds of colloidal substances tend to condense ions on or near their surfaces, because of their electrical properties, and that different kinds of ions may be condensed to different degrees. This means, of course, that when a solid substance is dispersed in a complex saline solution, the liquid phase, at a sensible distance from the solid particles, may

differ from the solution at the interphase boundary, both qualitatively and quantitatively. It appears that a sort of concentration gradient is set up between solid particles and the solution. One practical consequence of this differential absorption and condensation of ions at the interphase is that considerable indefiniteness must characterize any true statement that can now be made about the actual concentration of the ions that exist in the solution films surrounding the particles of alkali soils.

Theoretically, when evaporation brings about a reduction in the thickness of the films of solutions surrounding soil particles, not only does the average total concentration of the films increase, roughly inversely proportionally to the water content, but the percentage composition of the films tends to change also. Since evaporation and plant growth continually alter the water content of the soil, the so-called soil solution must necessarily be affected both qualitatively and quantitatively. There is, therefore, no such thing as a definite or true soil solution. Rather, the composition of the solution films present in soils changes more or less continually both qualitatively and quantitatively. This is true irrespective of the absorption of ions by plants but is, of course, profoundly affected by plant absorption.[1]

The effective concentration and composition of the solution films at the points of contact between absorbing roots and soil particles are difficult to determine for another reason, namely, because of the role played by the absorbed ions. It is well established that certain ions, when absorbed on the surface of colloids, can be taken up by plant roots. In fact, several workers have shown that absorbed K is readily available to plants. This means that an ion, absorbed on one type of colloid (the soil) can pass over to another type of colloid (the root surface). In this ion transfer an exchange undoubtedly takes place, probably between some one or more cations of the plant or H ions formed by the CO_2 given off by the roots and the ion on the colloid. Another difficulty is caused by carbonates and possibly other salts of low solubility. Where $CaCO_3$ is present in finely divided form, Ca ions probably continue to be brought into solution through the solvent action of CO_2 given off by growing roots.

It is also important to bear in mind that alkali soils are extremely variable (3). The content of a given salt at one point in the soil may be several fold what it is a few feet away. Not infrequently the Cl content of the soil is found to vary 10 to 20 per cent within a distance of 6 inches or less. The roots of a single plant growing in natural soil may be, therefore, in contact with widely different kinds of solutions.

In view of these facts, there seems to be no good reason for refinement, to

[1] It is true that the soil solution may be defined as that part of the liquid phase which is capable of more or less free movement, but even this definition has only limited value, since various factors exert an influence. If the term "soil solution" is to have definiteness, the conditions to which it is applied must be given as fully as possible.

an extreme degree, of methods for the determination of absorbed Na, of water-soluble Na, or of any other cation of alkali soils.

The state of knowledge is equally indefinite as regards the physical effects that are produced by small variations in the ratios of the absorbed ions. As is well known, marked physical effect is produced on soils by bringing about a high degree of Na saturation. Even this effect may be profoundly modified, however, by changing the concentration of soluble electrolyte present. For example, highly dispersed Na-saturated soil may be pronouncedly flocculated by the addition of a strong solution of NaCl.

When the percentage saturation with Na is comparatively low, say 5 to 10 per cent, it is difficult to determine what the physical effect of the absorbed Na really is. There are several reasons for this difficulty. First, the various methods by which any desired degree of Na saturation is effected alter the soil in other respects and especially as regards the concentration and composition of its soluble salts. The result is that the soil particles, after the desired degree of Na saturation has been effected, will be surrounded by a different kind of solution from that originally present. Secondly, the microbiology of the soil may also be altered to a marked degree, with the result that the soil suffers changes incident to subsequent microbiological activity. Thirdly, Na may exist in soils in different degrees of exchangeability. Many different kinds of solid substances have the power to absorb Na. Some substances attract Na ions much more strongly than do others. In such cases we would expect that the absorbed Na would produce different physical effects on the system as a whole. Moreover, Wiegner (10) showed that different parts of the surface of the same substance may attract a given kind of cation unequally. None of the methods ordinarily used for the determination of absorbed Na, however, differentiate between different kinds of absorbing surfaces, all those present being commonly treated as a unit. Furthermore, the various methods in use commonly dissolve more or less Na from water-insoluble substances.

For these and perhaps other reasons, there does not seem to be any good reason for the assignment of special significance to small differences in the Na content of alkali soils, whether it be the water-soluble or the absorbed form that is considered.

If the foregoing discussion is sound, then it follows that we are unable to assign definite values to those solution films of an alkali soil which are in contact with the roots of growing plants. Just what the effective concentration of Na, for example, actually is and its relation to the concentration of other ions, can be only roughly approximated at the present time. There is certainly no justification for the assumption that the ion population of the displaced solution of an alkali soil is the same in all important respects as that which exists at the soil particle-root contact.

As indicated already, the preceding data show that the displaced solutions of the two soils used in this investigation were approximately true aliquots, in so far as Cl is concerned, of the entire moisture content of these soils. This

agrees well with the conclusions of Burd and Martin (1). The situation, as regards SO_4 and cations, however, is not so simple. At the interphase boundary it is highly probable that the concentration and proportions of SO_4 and cations are substantially different from what they are in free solution of the soil, such as is obtained by displacement procedure. Since many alkali soils contain sulfates of low solubility and possibly of high adsorbability, either extraction with water or displacement may lead to very erroneous conclusions concerning the effective SO_4 concentration in the natural soil.

In view of the foregoing discussion, data on the salt tolerance of plants obtained by means of soil cultures can not now be interpreted strictly. Since the solid phase of the soil influences the composition and concentration of the liquid phase, and since the roots of growing plants affect the solid constituents of the soil, reliable information concerning the specific physiological effect of the different ions can be best obtained by means of solution cultures or possibly sand cultures. Certainly the elucidation of the physiological principles involved in salt tolerance requires the use of a medium for growth which is substantially different from that of ordinary soil.

When soil is used as a culture medium, particularly when plants are grown in the open field, the number of variables involved, the magnitude of which is difficult, if not impossible, to determine, is so great as to preclude the positive determination of the physiological principles. The variables include not only those already discussed, but also light intensity, temperature and atmospheric variables, free oxygen, the minor elements, pH, organic matter, microbiological activities, and moisture relation. Field experiments on salt tolerance, conducted in a given place, are, therefore, merely local tests, the direct applicability of which is limited to that particular locality and not even certainly to different parts of that locality, because of the extreme variability of alkali soils.

Obviously, the practical application of the physiological principles which govern salt tolerance requires the use of actual soil, but the determination of the principles is fundamental.

From the foregoing discussion it is also obvious that solution culture data cannot, at present, be applied directly to natural alkali soils. Even with homogeneous soil, which is, of course, never found in nature, the conditions which affect plant growth differ greatly from those of solution cultures. There is, therefore, a wide gap to be bridged between solution cultures and field soil cultures. The fact that solution culture data cannot now be confidently applied directly to practical alkali soils, is no reason, however, why solution cultures should be discouraged, for it is probably only by means of such cultures that the principles which control the growth of plants in saline soils can be elucidated, and it is important that the principles be fully understood. It is perhaps still more important that the soil be fully understood in its varied and complicated chemical, physical, and biological relationships.

The suggestion is made from time to time that displaced solutions of saline soils will be useful, either directly or indirectly, in the study of salt tolerance

of plants. It is a little difficult to see the logic of this idea. First, a solution of any composition and concentration is what it is irrespective of where or how it was obtained, and saline solutions can be prepared in the laboratory as desired without resorting to displacement procedure. Moreover, the extensive literature on alkali soils shows quite clearly that almost every conceivable composition and concentration of soluble salts are present in natural alkali soils. The variations are literally "all over the map." It is entirely unnecessary to go to the trouble of making displacements from soils in order to prove this point. Secondly, a displaced solution of a given soil in a strict sense merely represents itself. As shown already, it certainly is not the same, in all respects of importance to plant growth, as that at the soil-root contacts. Third, the foregoing data and discussion show that water extracts of soils, which are much easier to prepare than displaced solutions, probably give as nearly the truth about most of the salines of alkali soils as do displaced solutions. Fourth, a displaced solution of an alkali soil is almost certain to be an unbalanced and unfavorable medium for growth, except possibly for a very brief period. This is true not merely because the concentration of Cl, SO_4, HCO_3, Ca, Mg, or Na present may be so great as to necessitate dilution, but also because of the unduly low concentration of PO_4, NO_3, and K, to say nothing of the necessary minor elements. Dilution would, of course, accentuate the deficiencies in the last-named constituents, and to supply additional amounts of these would alter the displaced solution substantially. There appears to be no advantage to be derived, therefore, from the use of displaced solution in the study of salt tolerance of plants. This point is intended to apply particularly to alkali soils. The writer is well aware that much valuable information concerning soil processes and soil fertility has been obtained by researches on the so-called soil solution of nonalkali soils.

SUMMARY

The total amount of Na that is removed from an alkali soil by displacement procedure may be a few milliequivalents per 100 gm. less than that removed by 1 to 5 water extraction. This does not justify the conclusion that the exchangeable Na, as ordinarily determined, is seriously in error. The factors which influence the determination of exchangeable Na in alkali soils are so varied and numerous that a high degree of precision of method will probably serve no useful purpose.

Dilution appears to affect to less extent the determination of water-soluble Ca and Mg than that of Na. Among the common anions of alkali soils, the total amounts of HCO_3 and SO_4 found are likely to be the most markedly affected by dilution, whereas Cl was scarcely affected in the soils investigated. The same was probably also true of NO_3.

It is pointed out in this paper that the solution displaced from an alkali soil differs substantially from the solution at the growing root-soil particle contact. The bearings of this investigation on salt tolerance studies are discussed briefly.

REFERENCES

(1) BURD, J. S., AND MARTIN, J. C. 1923 Water displacement of soils and the soil solution. *Jour. Agr. Sci.* 13: 265–295.

(2) EATON, F. M., AND SOKOLOFF, V. P. 1935 Absorbed sodium in soils as affected by the soil-water ratio. *Soil Sci.* 40: 237–247.

(3) KELLEY, W. P. 1922 Variability of alkali soil. *Soil Sci.* 14: 177–189.

(4) KELLEY, W. P. 1934 The formation, evolution, reclamation and the absorbed bases of alkali soils. *Jour. Agr. Sci.* 24: 72–92.

(5) KELLEY, W. P. 1937 The reclamation of alkali soils. Calif. Agr. Exp. Sta. Bul. 617.

(6) KELLEY, W. P., AND BROWN, S. M. 1921 The solubility of anions in alkali soils. *Soil Sci.* 12: 261–285.

(7) KELLEY, W. P., AND BROWN, S. M. 1934 Principles governing the reclamation of alkali soils. *Hilgardia* 8: 149–177.

(8) THOMAS, E. E. 1936 Reclamation of white-alkali soils in the Imperial Valley. Calif. Agr. Exp. Sta. Bul. 601.

(9) VANSELOW, A. P. 1932 Equilibria of the base-exchange reactions of bentonites, permutites, soil colloids, and zeolites. *Soil Sci.* 33: 95–113.

(10) WIEGNER, G. 1935 Ionenumtausch und Struktur. *Proc. Third Internatl. Cong. Soil Sci.* 3: 5–28.

EFFECT OF ORGANIC MATTER ON THE WATER-HOLDING CAPACITY AND THE WILTING POINT OF MINERAL SOILS[1]

GEORGE J. BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication October 28, 1938

Experimental data on the effect of organic matter on the water-holding capacity and the wilting point of mineral soils are limited in number (4) and are contradictory. As a result, three different opinions exist on the subject: organic matter, because of its high water-holding capacity, will increase markedly the water-holding capacity of mineral soils; although organic matter does tend to increase the total water-holding capacity of mineral soils, it also increases their wilting point, the net result being that the amount of available water is not greatly affected (5); inasmuch as the organic matter content of most arable mineral soils is comparatively low, and it is difficult and uneconomical to increase it materially, organic matter does not play a very important role in the water-holding capacity of mineral soils.[2]

Since it has been found that the moisture equivalent as determined by the suction method approximates closely the field moisture capacity of soils (1, 6), including the sands, and since it is now possible to determine the wilting point of soils rapidly by means of the dilatometer method (2, 3), it was decided to investigate the effect of organic matter on the water-holding power and wilting point of different types of soil to which had been added various amounts of the same or different organic materials. It is the purpose of this report to present the experimental results obtained in the investigation.

MATERIALS AND PROCEDURE

The organic materials used consisted of muck, peat,[3] and decayed horse-cow manure. The actual organic matter content of these materials, as determined by the combustion method, was 40.5 per cent for the muck, 47.9 per cent for the peat, and 36.2 per cent for the manure. The mineral soils to which these materials were added are Plainfield sand, Miami sandy loam, Sturgis sandy loam, Janesville silt loam, Opahwa silt loam, Aiken clay loam, and Ontonagon clay. To a series of 100-gm. samples of soil, on the oven-dry basis, were added 0, 2, 4, 6, 8, 10, and 12 gm. respectively of the finely ground organic

[1] Authorized for publication as journal article No. 336, n. s., of the Michigan Agricultural Experiment Station (Soils Section).

[2] Veihmeyer, F. J. Storage of water in soils and its availability to plants. Paper presented at Ann. Meeting, Amer. Soc. Agron. 1937.

[3] The muck was a well-decomposed organic topsoil taken from a cultivated field; the peat was a very raw uncultivated soil of organic origin.

materials. After thorough mixing of the soil and organic material, the moisture equivalent, and hence the field capacity, and the wilting point of each mixture were determined. The moisture equivalent was determined by the suction method (1); and the wilting point, by the dilatometer method (2). In order that the water-holding capacity of each mixture might be reduced to a comparable volume basis, Büchner funnels of the same volume, 40 cc.,

TABLE 1

Effect of muck on the water-holding capacity (moisture equivalent) and wilting point of Sturgis sandy loam

MUCK ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	13.3	7.61	5.69	90.4	12.02	5.15	5.70
2	15.7	8.65	7.05	84.5	13.27	5.51	6.52
4	18.7	9.44	9.26	81.1	15.15	7.48	9.22
6	20.4	9.90	10.50	76.6	15.62	8.10	10.57
8	23.9	10.36	13.54	74.5	17.80	10.07	13.52
10	25.3	11.28	14.02	71.7	18.14	10.02	13.97
12	27.0	12.03	14.97	69.0	18.63	10.18	14.75

TABLE 2

Effect of muck on the water-holding capacity (moisture equivalent) and wilting point of Plainfield sand

MUCK ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	9.40	1.7	7.70	92.20	8.67	7.17	7.78
2	13.9	2.69	11.21	90.00	12.51	10.07	11.19
4	16.5	4.08	12.42	84.35	13.92	10.49	12.43
6	18.8	5.60	13.20	80.30	15.10	10.60	13.20
8	20.1	6.55	13.55	78.70	15.80	10.64	13.52
10	22.3	7.58	14.92	76.20	17.0	11.22	14.72
12	24.1	8.50	15.60	73.10	17.62	11.61	15.88

were used in each case, and after the soils were suctioned for a time and became settled, they were levelled off at the top.

EXPERIMENTAL RESULTS

The experimental results obtained showing the effect of organic matter on the water-holding capacity and wilting point of soils are presented in tables 1 to 12 inclusive. These results show that the addition of organic matter

increases the available water in soils. This is true whether the results are computed on the weight basis or on the volume basis. The increase in the available water is more marked in the light-textured soils than in the clays. In the typical case of Sturgis sandy loam, for instance, the available water increases from 5.45 per cent with no manure to 29.63 per cent with 12 per cent manure. In the case of Ontonagon clay, on the other hand, the available

TABLE 3

Effect of muck on the water-holding capacity (moisture equivalent) and wilting point of Opahwa silt loam, 0–6 inches

MUCK ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	21.70	7.8	13.90	78.30	16.99	9.99	12.76
2	24.28	8.5	15.78	70.13	17.03	11.07	15.78
4	25.98	9.3	16.68	68.58	17.82	11.45	16.70
6	27.20	10.1	17.10	67.18	18.26	11.47	17.07
8	28.00	10.6	17.40	63.95	17.92	11.81	18.47
10	31.50	11.4	20.10	62.43	19.68	12.57	20.14
12	34.2	12.1	22.10	59.55	20.35	13.15	22.08

TABLE 4

Effect of muck on the water-holding capacity (moisture equivalent) and wilting point of Miami sandy loam

MUCK ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	12.50	5.30	7.20	91.20	11.40	6.57	7.20
2	15.20	6.76	8.44	85.48	12.97	7.19	8.41
4	17.70	7.93	9.77	82.52	14.67	8.13	9.85
6	20.04	9.00	11.04	77.90	15.61	8.60	11.04
8	21.63	9.92	11.71	76.52	16.54	8.95	11.70
10	23.40	11.00	12.40	76.10	17.80	9.43	12.39
12	25.10	11.70	13.40	73.70	18.50	9.88	13.41

water increases only from 20.50 per cent with no manure to 25.43 per cent with 12 per cent manure. The possible explanations for the smaller increase of water-holding power and available water by the organic matter in the clays are that the water-holding power of the clays is already high, and that the volume-weight change of the clays is comparatively small.

Since the results show that the percentage of available water increases with the increase of organic matter in soils by about the same degree on both the

TABLE 5

Effect of muck on the water-holding capacity (moisture equivalent) and wilting point of Aiken clay loam, 0–15 inches

MUCK ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	34.20	16.65	17.55	61.70	21.08	10.81	17.52
2	35.00	17.17	17.83	60.85	21.29	10.95	18.00
4	35.80	18.05	17.75	60.20	21.57	10.70	17.78
6	37.50	18.83	18.67	58.82	22.01	10.93	18.58
8	39.40	19.80	19.60	57.90	22.83	11.73	20.26
10	41.70	20.40	21.30	56.65	23.55	11.99	21.16
12	43.20	21.51	21.69	55.22	23.84	12.03	21.79

TABLE 6

Effect of muck on the water-holding capacity (moisture equivalent) and wilting point of Ontonagon clay

MUCK ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	39.85	19.35	20.50	64.69	25.76	13.24	20.47
2	42.50	19.75	22.75	60.85	25.96	13.89	22.83
4	43.67	20.90	22.77	60.43	26.37	13.74	22.74
6	44.68	21.15	23.53	60.10	26.86	14.15	23.54
8	45.43	22.40	23.03	59.75	27.14	13.79	23.08
10	46.30	23.40	22.90	59.08	27.33	13.50	22.85
12	47.55	23.65	23.90	57.50	27.34	14.10	24.52

TABLE 7

Effect of peat on the water-holding capacity (moisture equivalent) and wilting point of Opahwa silt loam, 0–6 inches

PEAT ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	21.70	7.82	13.88	73.85	16.03	10.30	13.95
2	25.43	9.60	15.83	64.50	16.40	10.85	16.82
4	29.20	10.95	18.25	59.30	17.31	10.82	18.25
6	31.30	12.15	19.15	54.35	17.01	11.97	22.02
8	36.33	13.07	23.26	52.00	18.90	12.10	23.27
10	38.68	14.19	24.49	49.62	19.20	12.16	24.51
12	40.84	15.10	25.74	49.12	20.06	12.10	24.63

TABLE 8

Effect of manure on the water-holding capacity (moisture equivalent) and wilting point of Sturgis sandy loam

MANURE ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	13.3	7.85	5.45	90.42	12.02	4.92	5.44
2	18.1	8.92	9.18	79.50	14.39	7.24	9.11
4	23.4	10.09	13.31	72.50	16.96	9.54	13.16
6	26.7	10.96	15.74	67.92	18.12	10.68	15.72
8	32.2	11.73	20.47	62.65	20.17	12.79	20.42
10	38.3	12.50	25.80	60.42	23.14	15.57	25.77
12	43.4	13.77	29.63	57.90	25.14	17.17	29.66

TABLE 9

Effect of manure on the water-holding capacity (moisture equivalent) and wilting point of Janesville silt loam, 0–6 inches

MANURE ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	21.82	7.75	14.07	76.41	16.67	10.78	14.11
2	26.64	8.90	17.74	71.45	19.04	12.69	17.76
4	29.39	9.83	19.56	65.92	19.36	12.88	19.54
6	31.65	10.62	21.03	64.20	20.32	13.48	21.00
8	34.23	11.27	22.96	61.20	20.95	14.00	22.88
10	40.95	12.00	28.95	56.11	22.98	16.20	28.87
12	43.00	12.63	30.37	54.12	23.27	16.47	30.43

TABLE 10

Effect of manure on the water-holding capacity (moisture equivalent) and wilting point of Opakwa silt loam, 0–6 inches

MANURE ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	21.7	7.5	14.2	73.85	16.03	10.56	14.30
2	24.8	8.5	16.3	70.40	17.46	11.51	16.35
4	27.3	9.4	17.9	68.08	18.59	12.19	17.91
6	30.2	10.2	20.0	63.08	19.05	12.62	20.01
8	31.9	10.8	21.1	60.38	19.26	12.68	21.00
10	34.7	11.6	23.1	57.62	19.98	13.30	23.08
12	37.0	12.3	24.7	55.83	20.66	13.75	24.63

weight and the volume basis, it naturally follows that it is immaterial which basis is used in a study of the problem, since both bases show the same principle.

TABLE 11

Effect of manure on the water-holding capacity (moisture equivalent) and wilting point Aiken clay loam, 0–15 inches

MANURE ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	34.20	16.70	17.50	61.70	21.10	10.76	17.44
2	37.10	17.21	19.89	59.30	22.00	11.83	19.95
4	39.20	18.17	21.03	58.44	22.91	12.38	21.18
6	40.90	18.88	22.02	56.50	23.10	12.43	22.00
8	43.60	19.85	23.75	53.35	23.26	12.56	23.54
10	47.20	20.45	26.75	50.52	23.85	13.55	26.82
12	52.00	21.56	30.44	47.80	24.85	14.51	30.36

TABLE 12

Effect of manure on the water-holding capacity (moisture equivalent) and wilting point of Ontonagon clay

MANURE ADDED	WEIGHT BASIS			VOLUME BASIS IN 1 CUBIC FOOT			
	Water retained	Wilting point	Available water	Dry soil	Water contained	Available water contained	Available water
per cent	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
0	39.85	19.35	20.50	64.00	25.50	13.40	20.94
2	41.65	20.05	21.60	62.20	25.90	13.47	21.66
4	43.35	20.80	22.55	60.00	26.01	13.50	22.50
6	44.43	21.52	22.91	58.90	26.17	13.43	22.80
8	45.43	22.27	23.16	57.90	26.30	13.46	23.25
10	47.20	22.85	24.35	55.60	26.25	13.54	24.35
12	49.00	23.57	25.43	54.10	26.51	13.78	25.47

SUMMARY

An investigation was conducted to ascertain the effect of organic matter on the water-holding capacity and wilting point of soils, different organic materials and various types of mineral soils being used.

The experimental results obtained show that, when expressed on the percentage basis, organic matter increases markedly the available water in light soils, and to a less extent that in heavy soils. This is true whether the computation is based on the weight basis or the volume basis.

REFERENCES

(1) Bouyoucos, G. J. 1935 A comparison between the suction method and the centrifuge method for determining the moisture equivalent of soils. *Soil Sci.* 40: 165–171.

(2) Bouyoucos, G. J. 1936 The dilatometer method as an indirect means of determining the permanent wilting point of soils. *Soil Sci.* 42: 217–223.

(3) Bouyoucos, G. J. 1938 A mechanical device for determining the permanent wilting point of soils by means of the cohesion method. *Soil Sci.* 46: 331–335.

(4) Feustel, I. C., and Byers, H. G. 1936 The comparative moisture-absorbing and moisture-retaining capacities of plant and soil mixtures. U. S. Dept. Agr. Tech. Bul. 532.

(5) Huberty, M. R. 1936 Effect of soil characteristics on plant development in relation to water conservation. *Proc. Soil Sci. Soc. Amer.* 1: 439–446.

(6) Veihmeyer, F. J., and Hendrickson, A. H. 1931 The moisture equivalent as a measure of the field capacity of soils. *Soil Sci.* 32: 181–193.

SOME FACTORS INFLUENCING THE HEAT OF WETTING OF SOILS[1]

H. A. WADSWORTH[2]

Hawaii Agricultural Experiment Station

Received for publication October 31, 1938

The fact that heat is evolved when dry soils are wetted has long been recognized. Since the phenomenon is associated with the smaller fractions within the soil, the measurement of the heat evolved has been used to determine the percentage of the soil within the colloidal range of partial sizes (1). Gile (5) has shown a fair correlation between the heats of wetting of soil colloids and their chemical composition, and Anderson and Byers (2) have determined the heats of wetting for colloidal materials from certain major soil groups. Bouyoucos (4) found a fair correlation between the heats of wetting of soils and the relative amounts of water that failed to freeze in the dilatometer.

In general, heat of wetting determinations are made on oven-dry samples; in fact, only recently has attention been directed (6, 8) toward the fact that heat is evolved when soils with appreciable moisture contents are saturated, although Puri, Crowther, and Keen (7) pointed out this possibility from theoretical considerations in 1925. The suggestion (8) that the heat of wetting of a soil is correlated with moisture content of that soil through an exponential curve focuses attention on the details of the procedure when low moisture percentages are involved; the shape of the curve indicates that small and usually insignificant differences in moisture content, at the low end of the moisture scale, are responsible for wide variations in the heats of wetting. The present study was prompted by a desire to refine the experimental procedure to such a degree that the quantitative results might be used with increased confidence.

EQUIPMENT AND PROCEDURE

In all cases heat evolution was measured in a vacuum flask provided with a thick, tightly fitting cork. In the early apparatus a Beckman thermometer passed through this cork at the center; a glass stirring rod, bent to encircle the thermometer bulb, could be operated through a hole at one side. The total water equivalent of the calorimeter with its accessories was determined electrometrically (6). The specific heat of oven-dry soil was assumed to be 0.2 calorie

[1] Published with the consent of the acting director of the Hawaii Agricultural Experiment Station.

[2] Irrigation engineer.

per gram per degree centigrade. When moist soils were used, it was assumed that the moisture in the soil had a specific heat of 1.

Although radiation losses from the vacuum flask, when tightly stoppered, were negligible under the greatest temperature difference that was encountered, such losses became significant when the stirrer was added. These losses were adjusted by plotting temperature reading against time before and after the addition of the sample. The extrapolation of the lines through these points to the time of soil addition provided a good index of the time required for a complete wetting of the soil as well as a means of evaluating the radiation losses and the actual temperature increase.

Although every effort was made to bring the dry soil sample to the same temperature as the initial temperature of the water in the calorimeter, this was not always possible. In such instances the temperature of the soil was noted at the time of introduction, and the necessary adjustments were made during computation.

The necessity for such corrections was obviated by a further refinement involving the use of glass bombs which were filled with soil, sealed, and placed on a block in the calorimeter under a smashing blade which could be operated from outside. Stirring was accomplished by a modified hypodermic syringe which extended through the cork. When this device was used, corrections for radiation losses were highly essential.

Two soils were studied in considerable detail. One of these, a residual lateritic soil from Oahu, has already been described (8); the other was Superior clay loam imported for the purpose.

EFFECTS OF METHODS OF COOLING

The common procedure in determining heat of wetting involves the oven drying of a soil sample, cooling in a desiccator, and the addition of the soil to the calorimeter through a small funnel. In the present work samples of about 20 gm. of hot, freshly oven-dried soil were placed in test tubes and then tightly corked. Corks were waxed in place. The sealed tubes were then cooled. At the time of the determination of heat of wetting the calorimeter was opened, the tube quickly uncorked, and about half of the soil added to the calorimeter. The remainder of the soil was used for the determination of the moisture content. Weighings of the tube and its contents permitted an accurate determination of the amount of soil added to the calorimeter, by the method of difference, and permitted the interpretation of the heat evolution in terms of calories per gram of soil on the oven-dry basis.

Three lots of tubes were used. Two of these lots consisted of six pyrex test tubes each, of such size that approximately 20 gm. of freshly oven-dried soil filled each tube completely when the cork was inserted. The other lot consisted of six tubes, each of such dimension that a similar volume of soil occupied only about one-fifth of the capacity of the tube. In all cases the tubes were quickly loaded with hot soil and immediately corked.

One of the lots of small tubes was allowed to come to room temperature slowly and to stand at room temperature for 64 hours before the determination of the heat of wetting. The other two lots were cooled quickly in a water bath. The determination was made as soon as the calorimeter temperature had been reached by the cooling soil.

Results are as given in table 1.

The difference of 0.7 calorie per gram between the heats of wetting of large tubes and of small tubes, quickly cooled, may be considered as decidedly significant in view of the small probable errors involved. It is to be noted, however, that the lower average heat of wetting is associated with a slightly higher moisture content. It seems logical to assume that the adsorption of moisture during the operation of dividing the sample between its two uses is at least partly responsible for the result. Moreover, there is some evidence that the soils in the larger tubes adsorbed some moisture from the air in the tubes during the process of cooling. But, regardless of the causes for this discrep-

TABLE 1

Effect of size of cooling tube and history of cooling on the heat of wetting—Ewa soil

TREATMENT	NUMBER OF DETERMINATIONS	HEAT OF WETTING	MOISTURE
		cal./gm.	*per cent*
Small tubes—long cool	6	4.37 ± 0.03	0.25 ± 0.02
Small tubes—quick cool	6	4.35 ± 0.04	0.29 ± 0.01
Large tubes—quick cool	6	3.65 ± 0.09	0.33 ± 0.08

ancy, it seems evident that samples to be used for the determination of the heat of wetting of moisture-free soils must be handled with great care.

EFFECT OF TEMPERATURE ON HEAT OF WETTING

In most accounts of determinations of heat of wetting of soils, the temperature of the contents of the calorimeter at the beginning of the test is reported as being close to room temperature for obvious calorimetric reasons. Hence, most of the results available apply to determinations made at temperatures near 25°C.

It has recently been suggested (8) that the heat of wetting of a relatively dry soil might be a function of the surface force with which the moisture is held. Baver and Winterkorn (3), after working with extracted soil colloids saturated with hydrogen, report that the adsorptive capacity of these materials, at a given vapor pressure, decreases markedly with increases in temperature. From such evidence it might appear that the heat of wetting of a soil at a specified moisture content should vary with the temperature at which the determination was made.

This possibility was tested with the two widely differing soils which have been mentioned. When the local soil was used, a lot of oven-dry material

was mechanically turned in a tight box through which a stream of water-saturated air was forced. Twenty test tubes were filled when the soil moisture had been increased to about 3 per cent. Eight of these tubes were successfully used for the determination of the heat of wetting at a temperature of about 8° C., and ten were used at 40° C. All tubes were conditioned overnight in water baths held at the specified temperatures. Although the vacuum bottle used for the measurement of heat evolution was submerged to its mouth in a large calorimeter carrying water at the same temperature as that in the vacuum bottle, radiation losses through the cork and its accessories, because of the increased thermal gradient, made adjustment necessary. As usual, half of each sample was used in the calorimeter, and half for moisture determination. Results are given in table 2.

TABLE 2

Results of determinations of heats of wetting made at 8°C. and at 40°C.—Ewa soil

TEMPERATURE AT DETERMINATION	NUMBER OF DETERMINATIONS	HEAT OF WETTING	MOISTURE
°C.		*cal./gm.*	*per cent*
8	8	1.93 ± 0.10	2.94 ± 0.01
40	10	0.77 ± 0.05	3.31 ± 0.05
Difference		1.16 ± 0.12	0.37 ± 0.05

TABLE 3

Results of determinations of heats of wetting made at 8°C. and at 40°C.—Superior clay loam

TEMPERATURE AT DETERMINATION	NUMBER OF DETERMINATIONS	HEAT OF WETTING	MOISTURE
°C.		*cal./gm.*	*per cent*
8	11	0.99 ± 0.05	2.01
40	10	0.69 ± 0.04	2.01
Difference		0.30 ± 0.064	

It is evident that the difference in the heat of wetting between the two lots is statistically significant, but the difference in average moisture contents reduces the confidence that can be placed in the result. The same soil studied at 25° C. (8) gave a value of 1.35 calories per gram at 3.30 per cent moisture, and 1.52 calories per gram at 2.94 per cent moisture. It seems doubtful if the small differences in moisture content reported in table 2 resulted in the great difference in the heat of wetting which is reported.

More convincing results were obtained with Superior clay loam. Here the procedure was modified by the use of bombs. Twenty-one bombs were filled with stock soils which had been wetted to a moisture content of about 2 per cent. Samples for moisture content were taken after every third bomb was filled. These results showed no significant or consistent variation in moisture content during the filling operation. The average moisture content was 2.01

per cent with a small probable error. An additional safeguard was provided by setting aside alternately filled bombs for each of the two treatments. Thus, bombs 1, 3, 5, etc. were used for determinations of heat of wetting in the hot regime; 2, 4, 6, etc., were used for the cold regime.

Each bomb was placed on a brass smashing block on the bottom of the calorimeter containing 100 gm. of water and was allowed to come to thermal equilibrium. When this had been accomplished, the bombs were smashed and temperature rises noted. Continuous observation of temperature permitted the evaluation of radiation losses.

Results are shown in table 3.

DISCUSSION

Although it has long been recognized that oven-dried soils are highly hygroscopic, allowance for this property is rarely made during determinations of heat of wetting. In fact, this characteristic is so marked with perfectly dry soils that the simple opening of the soil tube for division of the sample between calorimeter and weighing bottle adds sufficient moisture to the soil to modify its heat of wetting. It would appear that the significance of this change depends on the original moisture content of the soil, the temperature and humidity of the air, and the speed with which the manipulations are made. In view of the great hygroscopic capacity of such materials, it is doubtful if any highly colloidal soil can be kept dry enough in anything but the best desiccators to permit subsequent determinations of the heats of wetting if quantitative values for moisture-free soils are required. Although the use of bombs which can be broken when thermal equilibrium is attained within the system is tedious, it does provide a means of obtaining results which is more nearly free from objection than are the conventional methods.

SUMMARY

Results of determinations of heats of wetting with two soils indicate that such values are of only qualitative interest unless unusual precautions are taken.

The conventional cooling of oven-dried samples in ordinary desiccators may permit the adsorption of enough water prior to the determination of the heat of wetting to reduce materially the value of the result.

The heat of wetting of a sample of soil at a specified moisture content appears to be a function of the temperature at which the determination is made. Increases of temperature at the time of determination result in decreases in the heat of wetting, and conversely.

REFERENCES

(1) ANDERSON, M. S. 1924 The heat of wetting of soil colloids. *Jour. Agr. Res.* 28: 927–935.

(2) ANDERSON, M. S., AND BYERS, H. G. 1931 Character of the colloidal material in the profiles of certain major soil groups. U. S. Dept. Agr. Tech. Bul. 228.

(3) BAVER, L. D., AND WINTERKORN, H. 1935 Sorption of liquids by soil colloids: II. Surface behavior in the hydration of clays. *Soil. Sci.* 40: 403–419.
(4) BOUYOUCOS, G. J. 1918 Relationship between the unfree water and the heat of wetting of soils and its significance. Mich. Agr. Exp. Sta. Tech. Bul. 42.
(5) GILE, P. L. 1925 The nature of colloidal soil material. *Colloid Symp. Monog.* 3: 216–227.
(6) HOSEH, M. 1937 Heat of wetting of some soil colloids at different moisture contents. *Soil Sci.* 43: 257–275.
(7) PURI, A. N., CROWTHER, E. M., AND KEEN, B. A. 1925 The relation between the vapour pressure and water content of soils. *Jour. Agr. Sci.* 15: 68–88.
(8) WADSWORTH, H. A. 1938 Some thermal phenomena in a selected Hawaiian soil. *Soil Sci.* 45: 251–262.

THE CONTROL OF NUT GRASS WITH CHLOROPICRIN[1]

G. H. GODFREY

Texas Agricultural Experiment Station

Received for publication October 18, 1938

The so-called nut grass, *Cyperus rotundus* (which is a sedge and not a grass), is an agricultural pest of serious importance in subtropical places such as Hawaii and the southern border of the United States from Florida to southern California. Aside from reproduction by seed, extensive vegetative spread and reproduction takes place by means of underground stems or rootstocks, portions of which become swollen with stored starch to form tubers some of which are an inch long and a half inch in diameter. These tubers are long-lived, which makes eradication of the weed by ordinary cultivation operations extremely difficult. Spread is rapid, and in many instances a small initial infestation in a field will spread in a year or two to cover several times the original area. Cultivated lands have been abandoned because of the encroachment of nut grass. The development of control measures for nut grass has been the objective of a number of agronomists who have recognized the seriousness of this pest. Smith and Fick (4) give a fairly complete life history of nut grass and its characters as related to its control. Smith and Mayton (5) show from their experimental work that under Alabama conditions, plowing or disking every 3 weeks during the growing season, April to October, for 2 years eradicated nut grass completely. Treatments during one season alone eradicated only about 80 per cent of it.

In Lower Rio Grande Valley and other subtropical localities in the United States and its territories where growing conditions prevail 12 months in the year, the eradication treatments would have to continue throughout the year, thus materially increasing the cost. Furthermore, under certain conditions it is highly desirable to eradicate the weed from limited areas of soil quickly in order to use the soil for other purposes. A quick, effective method of eradication, therefore, is highly desirable. .

An indication of excellent control of nut grass by chloropicrin fumigation of the soil has previously been mentioned (3) in connection with experiments on the control of root-knot nematodes in pineapples in Hawaii. In the spring of 1937 similar experiments were conducted at the Lower Rio Grande Valley Experiment Station at Weslaco, Texas, with nut grass control as the main objective. The ground in the station lath house had become so heavily infested with nut grass as to be useless for plant propagation purposes. In

[1] Published with the approval of the director as Contribution No. 474, Technical Series, of the Texas Agricultural Experiment Station.

a sample square yard area, 606 separate tillers were counted. An excellent site, therefore, was available for the experiments.

EXPERIMENTAL

Experiment 1. An area 6 x 21 feet was marked off and spaded. Chloropicrin was applied in dosages of about 3½ cc. in holes 12 inches apart to a depth of 7 inches (rate about 560 pounds per acre). The entire area was then covered with a large sheet of glue-coated gas-proof paper, the edges of which were buried to a depth of 6 inches in the soil and wetted down around the entire periphery of the plot. This method of treatment is essentially the same as that described by the author (2) for fumigation of soil for the control of soil fungi and by Godfrey and Young[2] for soil fumigation in general.

Four days after the treatment was applied, the odor of chloropicrin was still apparent through a break made in the paper. On May 28, one week after the treatment was applied, the paper was removed, and the odor of gas was still apparent.

On June 21, one month after the treatment was applied, 10 live nut grass plants had emerged within the treated area. Eight of these were near the edge and were definitely traced to encroachment into the area from live plants on the outside. Two were about 1½ feet from the edge and on exploration proved to be from tubers that had survived the treatment, one 20 inches and the other 21 inches below the surface of the soil. Examination of tubers found free in the soil showed the vast majority of them to be brown internally and dead. Figure 1, plate 1, shows, at the right, a healthy plant with its parent tuber, which is white in the interior; and at the left, the two surviving plants with tubers partly burned and a group of dead tubers, dark brown throughout. Counting the two plants in the interior of the plot as the sole survivors, the control was such that there was $\frac{1}{4}\frac{1}{0}$ of 1 per cent survival. Subsequent observations showed that no further plants developed within the treated area, except those encroaching from the growth of underground stems from living plants on the outside. Figure 2, plate 1, shows the treated strip of soil entirely devoid of plant growth in contrast with the heavy extent of nut grass in adjoining areas.

Experiment 2. On August 2, 1937, an additional experiment was begun as follows, each plot being 3 x 9 feet:

Plot 1. Chloropicrin applied 3½ cc. per injection at 12-inch intervals; rate 560 pounds per acre; cover, glue-coated mulch paper; edges buried 4 inches; edges wetted down.

Plot 2. Quantity per injection same as that in plot 1, with one-third more points of injection; rate 750 pounds per acre; paper similar to that in plot 1.

Plot 3. Rate reduced to 2½ cc. per injection, or 400 pounds per acre; paper similar to that in plots 1 and 2.

[2] Godfrey, G. H., and Young, P. A. Directions for the use of chloropicrin and carbon bisulphide as soil fumigants. Tex. Agr. Exp. Sta., Prog. Rpt. 514. (Mimeographed) Apr. 15, 1938.

Plot 4. Rate same as that in plot 3; cover, glue-coated Kraft paper, wetted down on the surface without burial of the edges.

Plot 5. Rate same as that in plots 3 and 4; no paper cover. Soil wetted down about 2 inches and renewed three times during the afternoon.

Plot 6. Spaded but untreated.

In addition to the six plots, a large area at one end of the lath house was treated as in plot 5; no cover, surface wetted down by sprinkling with hose.

Two weeks after treatments were applied, the coverings were removed and observations made as follows:

Plots 1 to 4 all showed dead grass with no sign whatever of green. In plot 5 the killing of plants was slower, but the leaves gradually died after turning yellow at their bases. On October 11, a little over 2 months after the treatments were applied, observations were made on the survival of nut grass in the treated plots. The results are shown in table 1. Control percentages are based on the round number of 500 tillers per square yard instead of 606 found in the one plot counted.

TABLE 1

Results in nut grass control by chloropicrin treatments

PLOT NUMBER	TREATMENT PER ACRE	COVER	SURVIVAL	
			Number	Per cent
	lbs.			
1	560	Mulch paper	0	0
2	750	Mulch paper	0	0
3	400	Mulch paper	10	0.67
4	400	Kraft paper	7	0.467
5	400	Wet soil	10	0.67
6	Check	None	About 375	25

All treatments gave practical control of nut grass. It is possible, by pouring a small quantity of chloropicrin around each surviving plant, to bring about its quick death. This chemical treatment, indeed, is probably preferable to the excavation of plants, since any breakage of the plant stem results in leaving some surviving part of the plant underground, whereas the chemical treatment, as applied, killed all remaining plants completely.

Observations on the large area treated similarly to plot 5 showed considerable survival of plants, probably due to inadequacy of watering after treatment and, therefore, to the too rapid escape of the gas from the soil. In order to complete the eradication of nut grass from the area, an additional treatment with chloropicrin was applied in areas of nut grass survival on October 11, shortly after a rainfall of about 1 inch which wetted the soil down to a depth of 5 or 6 inches. The applicator was adjusted for 8-inch depth injection. About 6 weeks later observation showed most of the plants to be completely killed. The relatively few surviving plants were in the upper layer of soil where they had been protected from the chloropicrin gas by the wet soil.

Such surviving plants having no living deep underground stems were readily removed by means of a hoe.

CONCLUSION

Chloropicrin fumigation has been demonstrated to be a very effective means of eradicating nut grass from the soil. It is probably too expensive for large-scale field application. [Here, the Smith and Mayton tillage method (5) is probably more practicable.] For small home gardens and flower beds and for areas of encroachment into large fields, however, it is completely practicable. The cost for 100 square feet is approximately $1 for chloropicrin and a few cents for a gas-proof paper for cover. Since chloropicrin is so deadly to plant life of all kinds, it must be remembered that it will kill desirable plants as well as weeds. It must not, therefore, be applied in the vicinity of living shrubs or trees in the garden or flower bed. Ground to be treated must be entirely free of living plants that have a definite value. It is possible, of course, to remove them temporarily from the ground with a view to replacing them after the soil has been sterilized.

Chloropicrin fumigation likewise rids the soil of injurious insects, nematodes, many weed seeds of various kinds, and even injurious soil fungi (1, 2). It leaves no injurious chemical residue in the soil and, in fact, seems to produce a stimulative effect on plant growth caused rather by a beneficial effect on the biologic balance in the soil than by any direct fertilizing effect.

REFERENCES

(1) Godfrey, G. H. 1935 Experiments on the control of the root-knot nematode in the field with chloropicrin and other chemicals. *Phytopath.* 25: 67–90.

(2) Godfrey, G. H. 1936 Control of soil fungi by soil fumigation with chloropicrin. *Phytopath.* 26: 246–256.

(3) Godfrey, G. H., Oliveira, J., and Hoshino, H. M. 1934 Increased efficiency of chloropicrin for nematode control with better confinement of the gas. *Phytopath.* 24: 1332–1346.

(4) Smith, E. V., and Fick, G. L. 1937 Nut-grass eradication studies: I. Relation of the life history of nut grass, *Cyperus rotundus* L., to possible methods of control. *Jour. Amer. Soc. Agron.* 29: 1007–1013.

(5) Smith, E. V., and Mayton, E. L. 1938 Nut-grass eradication studies: II. The eradication of nut grass, *Cyperus rotundus* L., by certain tillage treatments. *Jour. Amer. Soc. Agron.* 30: 18–21.

PLATE 1

Effect of Chloropicrin Fumigation on Nut Grass

Fig. 1. Nut grass from experiment 1. *Right*, normal young plant with normal tuber, white on the inside; *left*, two young plants arising from partly injured deep-lying tubers; *below*, dark brown tubers, completely killed by the chloropicrin fumigation.

Fig. 2. The chloropicrin-treated bed, about 1 month after treatment, with the original stand of nut grass in the foreground and background. In experiment 2, nut grass was eradicated from the entire area, which is now again in use for plant propagation purposes.

FIG. 1

FIG. 2

UNIV. OF MICH.

SOME CRITICAL STUDIES OF THE PHENOLDISULFONIC ACID METHOD FOR THE DETERMINATION OF NITRATES[1]

EMERY M. ROLLER AND NELSON McKAIG, JR.[2]

U. S. Department of Agriculture

Received for publication October 18, 1938

The phenoldisulfonic acid method has been used for several years to determine nitrates in green manuring and soil fertility studies on Norfolk coarse sand at the Sandhill Experiment Station near Columbia, S. C. Some phases of these investigations have been reported (1). During the course of these studies, difficulties with the procedure were encountered such as turbidity and color in the aqueous extracts, discoloration of solutions after the addition of phenoldisulfonic acid and ammonium hydroxide, loss of nitrates from extracts during the course of analysis, and variable results due to changes in the sample-water ratio and changes in time of contact of sample with water. This paper reports a critical study of the method and describes a procedure finally developed to eliminate these difficulties.

EXPERIMENTAL

The samples employed in these studies are as follows: Samples 1 and 3—soils from a field fertilizer experiment, collected at different times; sample 2—a soil from a lysimeter experiment; sample 4—a stable manure containing some admixed soil; and sample 5—a mixture of partly decomposed soybean hay and soil. The soil samples are Norfolk sand.

Clarifying the extract

One of the most common difficulties encountered is turbidity of the extract, which usually persists throughout the procedure if not eliminated at the beginning and which often makes difficult a color comparison between the unknown and the standard. Flocculating or clarifying agents which have been recommended to eliminate turbidity from an extract include calcium carbonate (6, 11), calcium oxide (7), potassium alum (9, 14), and a mixture of copper sulfate, calcium hydroxide, and magnesium carbonate (8).

Some of these, and others, were studied in this laboratory, samples 1, 2, 4, and 5 being used. In each test, 100 gm. of samples 1, 2, and 5, and 20 gm.

[1] A contribution from the Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the South Carolina Experiment Station cooperating at the Sandhill Experiment Station, Columbia, S. C. Acknowledgment is made to A. B. Bowen for laboratory assistance rendered during the progress of this work.

[2] Assistant chemist, and associate soil technologist, respectively.

of sample 4 were mixed separately with 200 ml. of distilled water and with a weighed or measured portion of the flocculent. They were shaken three times, at 1-minute intervals, and filtered through folded filter papers. The first 50 or more ml. coming through was poured back, and a clean receiver was substituted. After complete filtration, the degree of clarity of the various extracts was determined. The results are listed in table 1 in the order of efficiency of the several clarifying agents used. The relative efficiency of the various clarifying agents was similar for each of the samples studied. Treatment of soil with calcium hydroxide, magnesium oxide, calcium sulfate, and Harper's mixture (8) each produced clear filtrates, but all were colored except those from the calcium sulfate treatment, which had also no apparent effect on the color of the soybean manure mixtures, neither dissolving from the samples

TABLE 1

Relative efficiency of various materials as clarifying agents

CLARIFYING AGENTS	QUANTITY ADDED	CLARITY OF EXTRACTS	COLOR OF EXTRACTS
$CaSO_4$*	25 ml.	Clear	Colorless
$CuSO_4$, $Ca(OH)_2$, and $MgCO_3$ (8)	5 ml. *N* $CuSO_4$ 0.4 gm. $Ca(OH)_2$ 1.0 gm. $MgCO_3$	Clear	Yellow
$Ca(OH)_2$	1.0 gm.	Clear	Yellow
MgO	1.0 gm.	Clear	Yellow
Alumina cream	2 ml.	Slightly turbid	Colorless
$CaCO_3$	1.0 gm.	Slightly turbid	Colorless
Carbex†	1.0 gm.	Slightly turbid	Colorless
Darco†	1.0 gm.	Slightly turbid	Colorless
Norit†	1.0 gm.	Slightly turbid	Colorless
Hyflo‡	1.0 gm.	Turbid	Colorless
None		Turbid	Colorless

* 25 ml. of a saturated solution of calcium sulfate diluted to 200 ml. with distilled water used for extractions instead of distilled water.

† Commercial forms of carbon black.

‡ A commercial diatomaceous filter aid.

nor diminishing from the solutions color due to soluble organic matter. The color of the extracts was due, no doubt, to the action of the weak bases on the humic materials of the soils.

Loss of nitrates due to acidity of the extract

The necessity for neutralizing the acidity of an extract prior to evaporation was noticed by Chamot et al. (4), Davis (5), and Harper, all of whom recommended calcium hydroxide. These workers reported that nitrates were lost when phenoldisulfonic acid was added to a residue containing carbonates, but this could not be confirmed by Fraps and Sterges (7) or by the data in table 2.

Loss of nitrates caused by acidity of the extract was studied, the following nitrate-free reagents being used: phenoldisulfonic acid (4), a saturated solution

of calcium sulfate, a 1:1 solution of ammonium hydroxide, a 10 per cent solution of sodium hydroxide, a 20 per cent solution of sodium carbonate, and solid calcium hydroxide and calcium carbonate. These materials did not effect a theoretical recovery of nitrates from a standard solution.

Standard potassium nitrate solution was added to 100-gm. portions of samples 1 and 2 so that the nitrate concentrations ranged from that existing in the soil (sample 1, 0.22 p.p.m., and sample 2, 0.56 p.p.m.) to about 16 p.p.m. After the mixtures had dried, they were extracted according to the procedure previously described. The basic substances were then added to aliquots of each filtrate, the solutions evaporated to dryness, and nitrates determined.

The data in table 2 clearly show the necessity for adding a base to soil extracts before evaporation. The loss of nitrates when no base was added might be attributed to decomposition or evaporation of nitric acid formed by

TABLE 2

Effect of various bases on nitrate recovery from soil extracts

Average of samples 1 and 2

Nitrate nitrogen added ..... *p.p.m.*	None	0.50	1.00	2.00	4.00	8.00	16.00
Basic substances used*	Nitrate nitrogen recovery						
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
None	0.29	0.44	0.75	1.32	2.01	3.23	12.09
NH_4OH	0.24	0.42	0.69	1.05	2.56	3.55	13.13
NaOH	0.38	0.74	1.17	2.05	4.03	8.09	16.31
Na_2CO_3	0.38	0.76	1.17	2.01	3.94	7.98	16.13
$Ca(OH)_2$	0.38	0.73	1.11	2.03	4.00	7.93	16.34
$CaCO_3$	0.40	0.76	1.15	2.03	3.99	8.14	16.29

* The following amounts of basic substances were used: 1:1 NH_4OH—4 drops, 10 per cent NaOH solution—2 drops, 20 per cent Na_2CO_3 solution—2 drops, solid $CaCO_3$—0.05 gm., and solid $Ca(OH)_2$—0.05 gm.

the reaction of free acid and nitrate salts in the solution. When ammonium hydroxide was employed, the loss was due, perhaps, to the volatilization of ammonium nitrate during the last stage of evaporation. Although satisfactory results were usually obtained with sodium hydroxide, sodium carbonate, and calcium hydroxide, their use is not generally recommended because of their solvent action on humic materials. They usually gave colored residues from extracts, whereas calcium carbonate yielded a white residue in every instance.

Sample-water ratio and time of contact of sample with water

The sample-water ratio and time of contact of the sample with water were studied, sample 1 being used. The data in table 3 indicate that the most satisfactory soil-water ratio is 1:2 for this sample. There is little choice between the ratios for lower nitrate concentrations, but for higher concentra-

tions the 1:2 ratio shows the greatest recovery. As shown in table 4, 18 hours' contact of soil and water is associated with a loss of nitrates. There was little choice between the 5-minute, 0.5-hour, and 2-hour periods in the case of the higher concentrations; however, the 5-minute contact showed the best recovery with low concentrations.

TABLE 3

Effect of soil-water ratio on nitrate recovery

NITRATE NITROGEN ADDED*.....*p.p.m.*	0.50	1.00	4.00	8.00
Soil-water ratio†	Nitrate nitrogen recovery			
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1:1	0.61	1.04	3.78	8.00
1:2	0.63	1.08	3.83	8.16
1:4	0.61	1.04	3.71	7.55
1:8	0.61	1.04	3.60	7.27

* 100 gm. of sample 1 containing 0.22 p.p.m. nitrate nitrogen used.
† Suspensions filtered after ½ hour.

TABLE 4

Effect of time of contact of soil with water on nitrate recovery

NITRATE NITROGEN ADDED*.....*p.p.m.*	0.50	1.00	4.00	8.00
Time of standing†	Nitrate nitrogen recovery			
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
5 minutes	0.66	1.12	3.83	7.65
½ hour	0.62	1.06	3.83	7.65
2 hours	0.60	1.04	3.83	7.65
18 hours	0.52	0.88	3.44	6.68

* 100 gm. of sample 1 containing 0.22 p.p.m. nitrate nitrogen used.
† Suspensions contained 1:2 ratio.

Decolorizing the extract

The carbon blacks Carbex, Darco, and Norit, commonly used as decolorizing agents, were found to adsorb nitrates appreciably from aqueous solutions. These results were in agreement with those of Emerson (6), Lipman and Sharp (9), and Ashton (2). Schreiner and Failyer (13), however, recommended G Elf carbon black alone as a decolorizing agent, and Harper (8) advised its use in a mixture. Neither alumina cream (6) nor a mixture of alum and potassium hydroxide (14) produced colorless solutions. Potassium permanganate (15) rapidly destroyed color, but the procedure was long and laborious. Fraps and Sterges reported that bromine water gave low values, which were due, possibly, to the formation of $NOBr_3$ (12, pp. 572–573)

from the reaction between phenoldisulfonic acid, bromides, and nitrates in the residue.

Superoxol was found to decolorize dark brown extracts, but its high nitrate content, and a pink-to-brown color which developed when the phenoldisulfonic acid solution of the residue was neutralized with ammonium hydroxide, made its use prohibitive. Since all samples of this reagent obtained from several commercial sources contained 20 to 50 p.p.m. of nitrate nitrogen, it was purified as follows: a 100-ml. portion was made slightly basic[3] to methyl red with sodium hydroxide, to neutralize the nitric acid present, and was distilled under reduced pressure (15–60 mm.) in an assembly consisting of a condenser and distillation flask, with a ground glass connection between them, and a receiver with a paraffin-treated cork. The temperature of the condenser was maintained at or below 20°C. Glass beads were added to prevent bumping. The first one-fourth of the distillate was discarded, and the distillation was continued until about one-fourth remained. More superoxol was added to the remaining liquid, and the procedure was repeated until the required amount of hydrogen peroxide had been obtained. The liquid remaining in the flask was rejected. Whenever a subsequent test showed the presence of nitrates, the reagent was redistilled until pure. This operation did not appreciably change its percentage composition. A trace of sulfuric acid was added to stabilize the purified product.

Tests with this reagent and with the peroxides of sodium, calcium, and barium showed a pink-to-brown color similar to that shown by superoxol. It was evident, therefore, that hydrogen peroxide and peroxides of bases oxidize phenoldisulfonic acid, possibly to some quinone derivative the ammonium salt of which imparts a pink-to-brown color to the solution. Fraps and Sterges, while studying the effect of this oxidant on color elimination apparently encountered the same difficulty, but they offered no explanation for it. This coloration was encountered in this laboratory in 1933 and 1934 while nitrates were being determined in soil samples from a field which had received an application of hydrated lime at the rate of 300 pounds per acre in 1933. The difficulty, which at that time was not explained, was due, perhaps, to the action of superoxol on particles of hydrated lime which remained in the soil.

In the present investigations this coloration was avoided by heating the residue with a few drops of carbonated water, which decomposed any basic peroxides formed by the hydrogen peroxide treatment, and expelling the hydrogen peroxide by evaporation. During the last stage of evaporation, however, before the addition of carbonated water, spontaneous ignition of the organic residue was occasionally encountered. The intense heat resulting was thought to cause a loss of nitrates. Plice (10) apparently attempted to avoid this firing by evaporating the superoxol with ammonium hydroxide. Although this base accelerates the decomposition of hydrogen peroxide and

[3] This adjustment was made outside the flask.

yields a white residue without firing, it is oxidized to nitric nitrogen, as shown below, and hence it should not be used. Spontaneous ignition of the residue and discoloration of the solution were prevented by the following procedure: The colored extract was concentrated to approximately 5 ml., and slightly more hydrogen peroxide than is needed for decolorization was added. The solution was concentrated to about 2 ml., diluted with a few drops of carbonated water, and evaporated to dryness. This operation of decomposing the metallic peroxides and hydrogen peroxide was repeated if necessary. One can best ascertain when this decomposition is complete by observing the characteristics manifested by quantities of water and hydrogen peroxide when evaporated to dryness in contact with inorganic residues. This reagent when concentrated by evaporation exhibits different characteristics from those of water, but when decomposition is complete, it has all the properties of an aqueous solution.

The effect of varying quantities of hydrogen peroxide on the nitrate content of a standard solution was determined for each of the aforementioned basic substances. The recovery was almost theoretical except from solutions treated with ammonium hydroxide, where nitrates increased with each addition of the reagent. This apparent oxidation was further shown by similar tests of aqueous extracts from sample 5. In these, use of ammonium hydroxide gave high nitrate values. Ashton (2) avoided the oxidation by boiling his samples with magnesium oxide to expel ammonia before treating the residues with superoxol. Calcium carbonate gave white residues and consistent values. Sodium hydroxide, sodium carbonate, and calcium hydroxide each produced colored residues and lower and variable results.

The effects of increasing quantities of hydrogen peroxide on the nitrate nitrogen recovery from samples 4 and 5 were determined. The aqueous extract of sample 4 was intensely brown; an extract of sample 5 was less intense. Table 5 shows that less than 1.50 ml. of hydrogen peroxide for sample 4 and less than 1.25 ml. for sample 5 were insufficient for complete decolorization of the extracts. The brown solutions were very difficult to match with a yellow standard, hence those results are approximate. Readings could not be obtained for two of the solutions. It is concluded that satisfactory results cannot be obtained unless the residue has been sufficiently oxidized so that it is not turned brown by the action of phenoldisulfonic acid.

The data in table 6 show that low values were obtained when no base or when ammonium hydroxide was added to the extracts. Calcium carbonate, sodium carbonate, and sodium hydroxide each gave similar results. Calcium carbonate prevented a loss of nitrates when ammonium hydroxide was used to neutralize the extract, but extensive oxidation of ammonia to nitric nitrogen occurred. These observations are in agreement with previously discussed data. Since this table primarily shows that hydrogen peroxide does not affect the nitrate nitrogen recovery from colorless extracts when various bases are used as neutralizing agents, it can reasonably be expected that the nitrate

TABLE 5

Effect of increasing quantities of superoxol on the nitrate recovery from samples high in soluble organic matter

	SAMPLE 4								SAMPLE 5							
H_2O_2 added.....*ml.*	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00
Nitrate nitrogen recovered.....*p.p.m.*	180.0	250.0	300.0	321.4	375.0	450.0	450.0	450.0	?	?	3.96	4.31	4.71	4.71	4.71	4.71
Color of residue.....	Brown ——— decreases ——— → White								Brown ——— decreases ——— → White							
Color of solution used for colorimetric comparison.....	Brown ——— decreases ——— → Yellow								Brown ——— decreases ——— → Yellow							

nitrogen recovery from extracts of samples high in soluble organic matter would not be affected. The results obtained, therefore, for samples 4 and 5 were considered absolute values for these extracts.

Ashton (2) says that amino and amide nitrogen present in extracts high in soluble organic matter are oxidized to nitric nitrogen. This was tested by evaporating 0.5-gm. portions of several types of organic nitrogenous compounds with and without hydrogen peroxide in an aqueous solution made basic with

TABLE 6

Effect of superoxol on nitrate recovery from extracts of soil 3 treated with various bases

H_2O_2 ADDED.....*ml.*	None	1.5
Basic substances used*	Nitrate nitrogen recovery	
	p.p.m.	*p.p.m.*
None	1.43	1.39
NH_4OH	1.54	9.09
$CaCO_3$	1.78	1.78
$CaCO_3 + NH_4OH$	1.78	16.67
NaOH	1.81	1.78
Na_2CO_3	1.78	1.78

* The amounts of basic materials added were the same as those in table 2.

TABLE 7

Effect of superoxol on the oxidation of some nitrogenous organic compounds

ORGANIC COMPOUNDS	NITRATE NITROGEN FOUND IN 100 ML. OF EACH SOLUTION	
	No H_2O_2 added	H_2O_2 added
	p.p.m.	*p.p.m.*
Acetanilide	None	None
Methylene amino aceto nitrile	None	0.5
P-nitro aniline	1.0	2.5
Egg albumen	None	0.1
Gelatin	None	Trace
Pyridine	None	None
Diphenylamine	None	None
Ammonium thiocyanate	None	0.2
Urea	None	Trace
Casein	None	Trace

sodium hydroxide. The solutions were carefully evaporated to dryness twice, 1 ml. of hydrogen peroxide being used each time, and nitrates were determined.

The results shown in table 7 indicate that for the compounds tested organic nitrogen in any form other than the nitro is not appreciably affected by hydrogen peroxide. There was evidence of very little hydrolysis or splitting and oxidation to nitric nitrogen, but since the quantity of nitrogenous organic compounds usually found in an aqueous extract of a sample high in soluble

organic matter is comparatively small, an error in the nitrate determination from this source would be negligible.

Ammonium salts, which might be found in some extracts, could be decomposed and the ammonia eliminated by concentrating the solution with magnesium oxide in lieu of calcium carbonate before treatment with H_2O_2.

REVISED ANALYTICAL PROCEDURE

Based on the several experiments discussed above, the following procedure has been found to give satisfactory results:

Reagents

(a) *Calcium sulfate solution.* Prepare saturated solution nitrate-free.

(b) *Phenoldisulfonic acid reagent.* Dissolve 25 gm. of pure white phenol in 225 ml. of concentrated sulfuric acid (sp. gr. 1.84), mix thoroughly, and heat for 6 hours at 100°C. in a lightly stoppered flask. Cool and store in an amber bottle.

(c) *Ammonium hydroxide.* Dilute the c.p. reagent 1:1 with water.

(d) *Standard nitrate solution.* Dissolve 0.7218 gm. of pure dry potassium nitrate in distilled water and dilute to 1 liter. One milliliter of this solution contains 0.1 mgm. of nitrate nitrogen.

(e) *Standard colorimetric solution.* Evaporate 10 ml. of solution (d) to dryness, cool, and dissolve in 2 ml. of reagent (b). After 5 minutes, dilute to 1 liter with distilled water. One milliliter of this standard contains 0.001 mgm. nitrate nitrogen, or 1 p.p.m.

(f) *Concentrated hydrogen peroxide.* Test for the presence of nitrates in hydrogen peroxide or superoxol by using the procedure below. If nitrates are present, distill as indicated in text.

(g) *Solid calcium carbonate.* Secure calcium carbonate nitrate free.

(h) *Carbonated water.* Saturate distilled water with pure carbon dioxide and store in an atmosphere of CO_2.

Preliminary procedure

Pour a mixture of 175 ml. of distilled water and 25 ml. of a saturated solution of calcium sulfate into a wide-mouth flask containing 100 gm., or less if the nitrate content is abnormally high, of the air-dried sample. Shake three times at 1-minute intervals and transfer the mixture to a folded filter paper. When the filtrate begins to come through clear, pour it back, being careful to avoid disturbing the filter mat, then permit the entire suspension to filter into a clean receiver. Measure an aliquot portion of this extract for analysis.

Procedure for colorless extracts

Evaporate the colorless aliquot to dryness in a beaker with about 0.05 gm. of $CaCO_3$. Avoid charring. Cool, and add to the residue 2 ml. of phenoldisulfonic acid, and stir with a glass rod until solution is complete. Dilute

the acid solution with about 50 ml. of water, and add ammonium hydroxide with constant stirring until alkaline. The formation of a yellow color at this point indicates the presence of nitrates. Dilute this solution to a definite volume and compare it colorimetrically with a standard diluted to an equal volume.

Procedure for colored extracts

Add to an aliquot portion of the colored extract in a beaker 0.05 gm. $CaCO_3$, or more if the acidity of the solution and hydrogen peroxide warrants, and concentrate to about 5 ml. Add to this liquid 1.5 ml. H_2O_2, and after further concentration, if the solution has not decolorized, add a few drops more. Repeat until the extract is decolorized. Evaporate the colorless solution to about 2 ml., dilute with a few milliliters of carbonated water, and again concentrate to about 2 ml. Continue this operation of dilution and concentration until the H_2O_2 has decomposed as indicated by text. Usually one dilution and evaporation is sufficient. Finally, evaporate the solution to complete dryness, being careful not to char any organic matter which has not been oxidized. From this point proceed as with colorless extracts.

The above procedure for the determination of nitrates does not provide for the removal of chlorides.

SUMMARY

Some difficulties in the colorimetric determination of nitrate nitrogen by the phenoldisulfonic acid method are critically examined.

Clarification of the extract was accomplished most satisfactorily with calcium sulfate solution.

The addition of calcium carbonate to the extract before evaporation, prevented a loss of nitrates due to acidity. It was the most satisfactory of all basic materials tried. Ammonium hydroxide caused a loss of nitrates.

The most satisfactory sample-water ratio was 1:2 by weight.

The best time of contact of sample with water was 5 minutes.

Concentrated hydrogen peroxide was the most effective decolorizing agent. Since commercial superoxol generally contains nitrates, it was purified by vacuum distillation. When hydrogen peroxide was heated with common bases, peroxides were formed. These reacted with phenoldisulfonic acid, producing a pink-to-brown color upon neutralization of the solution with ammonium hydroxide. The color was prevented by decomposing the peroxides of the residue with carbonated water and evaporating to dryness to expel all H_2O_2 vapor before adding phenoldisulfonic acid. Spontaneous ignition of the residue, which is likely to occur when the extract is evaporated to dryness with hydrogen peroxide, and discoloration of the solution were prevented by treatment with carbonated water. Calcium carbonate was the only basic substance tested which produced a white residue and did not form a peroxide.

Concentrated hydrogen peroxide oxidized ammonium hydroxide to nitrate but did not appreciably oxidize the nitrogen in several types of nitrogenous organic compounds.

A modified procedure for the determination of nitrate nitrogen by the phenoldisulfonic acid method is given.

REFERENCES

(1) Adams, J. E., Roller, E. M., and Boggs, H. H. 1936 A green manure fertilizer study on Norfolk sand. *Soil Sci.* 42: 175–185.

(2) Ashton, F. L. 1935 A rapid colorimetric method for determining nitrate nitrogen in grass. *Jour. Soc. Chem. Indus.* 2nd 54: 389–390T.

(3) Association of Official Agricultural Chemists 1925 Official and Tentative Methods of Analysis, ed. 2, p. 182. Washington, D. C.

(4) Chamot, E. M., Pratt, D. S., and Redfield, H. W. 1911 A study of the phenoldisulfonic acid method for the determination of nitrates in water. *Jour. Amer. Chem. Soc.* 33: 381–384.

(5) Davis, C. W. 1917 Studies on the phenoldisulfonic acid method for determining nitrates in soils. *Jour. Indus. and Engin. Chem.* 9: 290–295.

(6) Emerson, P. 1921 The colorimetric determination of soil nitrates in a colored water extract. *Soil Sci.* 12: 413–417.

(7) Fraps, G. S., and Sterges, A. J. 1931 Estimation of nitric and nitrous nitrogen in soils. Tex. Agr. Exp. Sta. Bul. 439.

(8) Harper, H. J. 1924 The accurate determination of nitrates in soils—phenoldisulfonic acid method. *Indus. and Engin. Chem.* 16: 180–183.

(9) Lipman, C. B., and Sharp, L. T. 1912 Studies of the phenoldisulfonic acid method for determining nitrates in soils. *Univ. Calif. Pubs., Agr. Sci.* 1: 21.

(10) Plice, M. J. 1932 The determination of nitrates in soils containing soluble organic matter. *Soil Sci.* 33: 213–215.

(11) Potter, R. S., and Snyder, R. S. 1915 The determination of nitrates in soils. *Jour. Indus. and Engin. Chem.* 7: 863–864.

(12) Roscoe, H. E., and Schorlemner, C. 1920 A Treatise on Chemistry, ed. 5, vol. 1. Macmillan and Co., Ltd., London.

(13) Schreiner, O., and Failyer, G. H. 1906 Colorimetric turbidity, and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31.

(14) Shmuk, A. 1931 Determination of the nitrate content of plants. *Gos. idarstv. Institut Tobakovedeniya (State Inst. for Tobacco Culture) Bul.* 78: 7–19.

(15) Syme, W. A. 1909 The colorimetric determination of nitrates in soil solutions containing organic matter. *Jour. Indus. and Engin. Chem.* 1: 188–189.

COMPARISON OF METHODS OF DETERMINING SMALL QUANTITIES OF HCN

O. H. COLEMAN AND ROBERT GARDNER

Colorado Agricultural Experiment Station

Received for publication November 11, 1938

An investigation of the various methods of estimating small quantities of HCN was necessary in a recent study of cyanogenesis in Sudan grass.

Other studies of these methods have been made during the last few years. Smith (7) reported a comparison of the old alkaline titration method with the acid titration method, a gravimetric method of Clifcorn, and the prussian blue method of determining prussic acid in plant material. He found that the alkaline method was most variable, because the end point was hard to determine, and, consequently advised dropping this method from the official list. He advised further study of the other methods.

Later, Greene and Williams (5) reported a comparison of their modified alkaline titration method (1) with the prussian blue method, the thiocyanate method, the old alkaline method, and the photoelectric method of Bartholomew and Raby (2). They found that the prussian blue method was unreliable, and the adjustments of pH were difficult. They also objected to the method because some of the work had to be done under reduced pressure. The intensity of the red thiocyanate was greater than the intensity of the prussian blue. They recommended that the modified alkaline method be tentatively adopted, that the photoelectric method be adopted as official, and that autolysis be conducted with the distilling apparatus completely connected and enough water added to complete the distillation. They advised that further study be made of the colorimetric methods.

Greene and Breazeale (4) recommended that the modified alkaline method and the photoelectric method be adopted as official, that the prussian blue method be deleted, and that the present colorimetric methods be given no further study, since they require more manipulation than the suggested official methods. These investigators reported that the results by the thiocyanate method were lower than those by the modified alkaline method, probably because some of the HCN did not change over to NaCNS. The sulfur was difficult to remove.

EXPERIMENTS

During the summers of 1936 and 1937, several investigations were made concerning the various methods of estimating small quantities of HCN. The thiocyanate red method as reported by Johnson (6) and the modified alkaline

method (1) were used as described, except that all titrations in connection with the latter were made of the total distillates (125–150 cc.) instead of aliquots. Collecting more than 150 cc. of distillate was considered unnecessary, since in a series of eight samples of Sudan grass about 96.5 per cent as much HCN was obtained in the first 100 cc. as in a total of 200 cc. of distillate. Good results in titrating the total distillate were obtained at concentrations of 400 p.p.m. HCN, or less, in the green plant material.

Table 1 gives the results of a comparison of the accuracy of the prussian blue, thiocyanate red, and the modified alkaline methods of determining small quantities of HCN in solution. Pure chemicals were used for the tests, but since the transversion of KCN to KCNS introduces an error, pure KCNS was used instead of KCN for the thiocyanate red test. The prussian blue test is

TABLE 1

Comparison of the accuracy of the prussian blue, thiocyanate red, and modified alkaline methods of estimating small quantities of HCN

PRUSSIAN BLUE		THIOCYANATE RED		MODIFIED ALKALINE	
By analysis—HCN in solution	Actual HCN in solution	By analysis—HCN in solution	Actual HCN in solution	By analysis—HCN in solution	Actual HCN in solution
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
281	206	216	207	..	..
128	103	106	104	83	82
52	52	53	52	42	39
23	26	26	26	21	20
2	13	13	13	10	9
0	6	6	6	5	5
0	3	3	3	3	2
0	2	2	2	..	..
0	1	1	1	1	1
X^2..... 55.0276		0.4490		0.7072	
P..... Very small		> 0.99		> 0.98	

accurate through a very small range, whereas the other methods are fairly accurate throughout the range studied, as is shown in table 1 by the high X^2 (3) value of 55.0276 for the blue method and the very low X^2 values for the other methods.

Further studies were made of the thiocyanate red and the modified alkaline method to determine, if possible, which of the two methods gave the better results. A simple factorial design was used, but because of lack of homogeneity the data were assembled for each concentration separately, and statistical interpretations were made on that basis.

Table 2 gives the results of comparisons of the thiocyanate red and alkaline methods of analysis, using known quantities of KCN in solution and in distillates from a bulk sample of Sudan grass. It is evident that the modified

alkaline method gives a more nearly accurate determination of pure chemicals in solution, since, in all cases of known concentration, the results are much nearer the actual value and all differences between the methods are greater than twice the standard error of a difference. The thiocyanate results are consistently low. The differences in HCN in Sudan grass, found by the two methods, are within the error attributable to sampling fluctuations.

The distribution of variance due to "error" in analyzing by the thiocyanate red and modified alkaline methods is given in table 3. The "error" variance

TABLE 2

A comparison of the thiocyanate red and modified alkaline methods of estimating HCN solution of pure chemicals and distillations from bulk Sudan grass, 1937

METHOD	NUMBER OF SAMPLES	PURE CHEMICALS—KCN ESTIMATED FROM			BULK SUDAN GRASS—ESTIMATED HCN
		5 mgm. KCN in solution	25 mgm. KCN in solution	50 mgm. KCN in solution	
		mgm.	*mgm.*	*mgm.*	*p.p.m.*
Thiocyanate red	10	3.545	18.338	28.107	86.7
Modified alkaline	10	4.722	23.490	46.816	89.6
2 × S.E. of difference		0.400	2.390	4.600	5.2

TABLE 3

A comparison of the variances attributed to "error" of the thiocyanate red and modified alkaline methods of estimating small amounts of HCN in solution

METHOD	D.F.	PURE CHEMICALS						BULK SUDAN GRASS—HCN	
		5 mgm. KCN in solution		25 mgm. KCN in solution		50 mgm. KCN in solution			
		"Error" variance	*F*	"Error" variance	*F*	"Error" variance	*F*	"Error" variance	*F*
								p.p.m.	*p.p.m.*
Thiocyanate red	8	0.0750	....	6.7461	17.04*	26.6361	31.41*	56.96	4.41†
Modified alkaline	8	0.1250	1.67	0.3958	.....	0.8480	.....	12.91	....

* > 1 per cent point.
† > 5 per cent point.

of the thiocyanate red method is greater than that of the modified alkaline method in all cases, except the 5 mgm. KCN solution, whether the HCN in pure chemicals or that in plant material is determined. The *F* values (8) of 17.04 and 31.41 for pure chemicals (25 mgm. KCN and 50 mgm. KCN) are well above the 1 per cent point, and the *F* value of 4.41 is greater than that required for the 5 per cent point. The modified alkaline method, therefore, gives a more reliable measure of HCN both in pure chemical solutions and in plant material.

method (1) were used as described, except that all titrations in connection with the latter were made of the total distillates (125–150 cc.) instead of aliquots. Collecting more than 150 cc. of distillate was considered unnecessary, since in a series of eight samples of Sudan grass about 96.5 per cent as much HCN was obtained in the first 100 cc. as in a total of 200 cc. of distillate. Good results in titrating the total distillate were obtained at concentrations of 400 p.p.m. HCN, or less, in the green plant material.

Table 1 gives the results of a comparison of the accuracy of the prussian blue, thiocyanate red, and the modified alkaline methods of determining small quantities of HCN in solution. Pure chemicals were used for the tests, but since the transversion of KCN to KCNS introduces an error, pure KCNS was used instead of KCN for the thiocyanate red test. The prussian blue test is

TABLE 1

Comparison of the accuracy of the prussian blue, thiocyanate red, and modified alkaline methods of estimating small quantities of HCN

PRUSSIAN BLUE		THIOCYANATE RED		MODIFIED ALKALINE	
By analysis—HCN in solution	Actual HCN in solution	By analysis—HCN in solution	Actual HCN in solution	By analysis—HCN in solution	Actual HCN in solution
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
281	206	216	207	..	..
128	103	106	104	83	82
52	52	53	52	42	39
23	26	26	26	21	20
2	13	13	13	10	9
0	6	6	6	5	5
0	3	3	3	3	2
0	2	2	2	..	..
0	1	1	1	1	1
X^2..... 55.0276		0.4490		0.7072	
P..... Very small		> 0.99		> 0.98	

accurate through a very small range, whereas the other methods are fairly accurate throughout the range studied, as is shown in table 1 by the high X^2 (3) value of 55.0276 for the blue method and the very low X^2 values for the other methods.

Further studies were made of the thiocyanate red and the modified alkaline method to determine, if possible, which of the two methods gave the better results. A simple factorial design was used, but because of lack of homogeneity the data were assembled for each concentration separately, and statistical interpretations were made on that basis.

Table 2 gives the results of comparisons of the thiocyanate red and alkaline methods of analysis, using known quantities of KCN in solution and in distillates from a bulk sample of Sudan grass. It is evident that the modified

alkaline method gives a more nearly accurate determination of pure chemicals in solution, since, in all cases of known concentration, the results are much nearer the actual value and all differences between the methods are greater than twice the standard error of a difference. The thiocyanate results are consistently low. The differences in HCN in Sudan grass, found by the two methods, are within the error attributable to sampling fluctuations.

The distribution of variance due to "error" in analyzing by the thiocyanate red and modified alkaline methods is given in table 3. The "error" variance

TABLE 2

A comparison of the thiocyanate red and modified alkaline methods of estimating HCN solution of pure chemicals and distillations from bulk Sudan grass, 1937

METHOD	NUMBER OF SAMPLES	PURE CHEMICALS—KCN ESTIMATED FROM			BULK SUDAN GRASS—ESTIMATED HCN
		5 mgm. KCN in solution	25 mgm. KCN in solution	50 mgm. KCN in solution	
		mgm.	*mgm.*	*mgm.*	*p.p.m.*
Thiocyanate red	10	3.545	18.338	28.107	86.7
Modified alkaline	10	4.722	23.490	46.816	89.6
2 × S.E. of difference		0.400	2.390	4.600	5.2

TABLE 3

A comparison of the variances attributed to "error" of the thiocyanate red and modified alkaline methods of estimating small amounts of HCN in solution

METHOD	*D.F.*	PURE CHEMICALS						BULK SUDAN GRASS—HCN	
		5 mgm. KCN in solution		25 mgm. KCN in solution		50 mgm. KCN in solution			
		"Error" variance	*F*	"Error" variance	*F*	"Error" variance	*F*	"Error" variance	*F*
								p.p.m.	*p.p.m.*
Thiocyanate red	8	0.0750	....	6.7461	17.04*	26.6361	31.41*	56.96	4.41†
Modified alkaline	8	0.1250	1.67	0.3958	.....	0.8480	.....	12.91	....

* > 1 per cent point.

† > 5 per cent point.

of the thiocyanate red method is greater than that of the modified alkaline method in all cases, except the 5 mgm. KCN solution, whether the HCN in pure chemicals or that in plant material is determined. The *F* values (8) of 17.04 and 31.41 for pure chemicals (25 mgm. KCN and 50 mgm. KCN) are well above the 1 per cent point, and the *F* value of 4.41 is greater than that required for the 5 per cent point. The modified alkaline method, therefore, gives a more reliable measure of HCN both in pure chemical solutions and in plant material.

A comparison was made between the use of Kjeldahl distilling equipment with tin condensors and all-glass distilling equipment, since there was a possibility that some of the HCN might be retained on the tin as insoluble tin cyanide. Table 4 gives the results of this study. Slightly more cyanide was recovered through the all-glass than the part-tin distilling equipment from all concentrations of KCN and from Sudan grass, but the differences are within the limits ascribable to chance fluctuations.

TABLE 4

A comparison of the use of part-tin and all-glass distilling equipment in estimating small amounts of HCN in distillates of pure chemicals and bulk Sudan grass, 1937

DISTILLING EQUIPMENT	NUMBER OF SAMPLES	PURE CHEMICALS—KCN ESTIMATED FROM			BULK SUDAN GRASS—ESTIMATED HCN
		5 mgm. KCN in solution	25 mgm. KCN in solution	50 mgm. KCN in solution	
		mgm.	*mgm.*	*mgm.*	*p.p.m.*
Tin	10	3.962	20.418	36.323	85.9
Glass	10	4.305	21.410	38.600	90.5
2 × S.E. of difference		0.400	2.390	4.600	5.2

TABLE 5

A comparison of autolysis of green Sudan grass material in a closed Erlenmeyer flask with that in a closed Kjeldahl flask and with that in a Kjeldahl flask connected completely for distillation

DUPLICATE SAMPLES FROM 12 INBRED LINES OF SUDAN GRASS			DUPLICATE SAMPLES FROM 12 OTHER INBRED LINES OF SUDAN GRASS		
Method of autolysis	Average HCN	Number of samples	Method of autolysis	Average HCN	Number of samples
	p.p.m.			*p.p.m.*	
Erlenmeyer	140.7	12	Erlenmeyer	151.8	12
Kjeldahl	143.0	12	Kjeldahl, connected	152.3	12
2 × S.E. of difference	7.6	..		6.7	..

If autolysis of the plant material is carried on with all equipment connected for distillation, then the number of determinations in one day is limited to the number of individual distillation units available. Since more than 100 distillations per day were required for the best results, a study of conditions of autolysis was made. The samples from two sets each of 12 inbred lines of Sudan grass were used. For one set, one sample from each line was allowed to autolyze in a 500-cc. Erlenmeyer flask sealed with a rubber stopper, while the autolysis of another sample from each set took place in a Kjeldahl flask sealed with a rubber stopper. The other set compared autolysis in an Erlenmeyer with autolysis in a Kjeldahl flask with all distilling connections intact.

All distillations were made with a Kjeldahl apparatus. Table 5 gives the results of these studies. The differences in both cases in HCN were less than twice the standard error of a difference; consequently, the differences can be attributed to sampling fluctuations. No measurable loss, therefore, of HCN occurred during the transfer of the autolyzed material from an Erlenmeyer flask to the Kjeldahl flask.

CONCLUSIONS

Of the methods studied, the modified alkaline method of determining small quantities of HCN gave the most reliable results.

The tin condensor is satisfactory for distillations of solutions of HCN.

Autolysis in an Erlenmeyer with the resultant transfer of plant material to the distilling flask causes no apparent loss in HCN if the transfer is made with a reasonable amount of care.

Good results were obtained by the modified alkaline method by titrating the entire distillate instead of an aliquot.

REFERENCES

(1) Association of Official Agricultural Chemists 1936 Alkaline titration method. *Jour. Assoc. Off. Agr. Chem.* 19: 94.

(2) Bartholomew, E. T., and Raby, E. C. 1935 Photronic photoelectric turbidimeter for determining hydrocyanic acid in solutions. *Indus. and Engin. Chem., Analyt. Ed.*, 7: 68–69.

(3) Fisher, R. A. 1930 Statistical Methods for Research Workers, ed. 3. Oliver and Boyd, Edinburgh, London, etc.

(4) Greene, R. A., and Breazeale, E. L. 1937 Report on hydrocyanic acid in glucoside-bearing materials. *Jour. Assoc. Off. Agr. Chem.* 20: 444–447.

(5) Greene, R. A., and Williams, J. A. 1936 Report on hydrocyanic acid in glucoside-bearing materials. *Jour. Assoc. Off. Agr. Chem.* 19: 589–594.

(6) Johnson, M. O. 1916 On the determination of small quantities of hydrocyanic acid. *Jour. Amer. Chem. Soc.* 38: 1230–1235.

(7) Smith, G. 1934 Report on hydrocyanic acid in glucoside-bearing materials. *Jour. Assoc. Off. Agr. Chem.* 17: 182–185.

(8) Snedecor, G. W. 1934 Calculation and Interpretation of Analysis of Variance and Co-variance. Collegiate Press Inc., Ames, Iowa.

PHYSICAL CHARACTERISTICS OF SOILS: III. HEAT OF WETTING

AMAR NATH PURI AND R. C. HOON

Irrigation Research Institute, Lahore, India

Received for publication December 7, 1937

The mechanism of moisture absorption by soils is not well understood. Crowther and Puri (2) brought forth evidence which seemed to indicate that the moisture in soils was most probably held in minute capillaries; these micropores are of the order of 1 $\mu\mu$ in diameter. The micropore hypothesis has an attractive simplicity, but the evidence in its favor is still insufficient. In the elucidation of the exact nature of the soil moisture relationships, measurements of the heats of wetting are likely to prove very useful.

Heat of wetting of soils has been measured by several workers (1, 3, 4), but the measurements have been confined chiefly to the total heat of wetting. Very little is known about the heat of wetting when soil is allowed to absorb increasing amounts of water. It seems reasonable to suppose that the greatest heat of wetting will be produced in the initial stages and that it will gradually become less as the soil takes more and more water. The difficulty of measuring heat of wetting with gradual increments of moisture is very great. For uniformity of results, the absorption must be allowed to take place in the vapor phase, and it is not possible to measure the rise of temperature accompanying the slow absorption of moisture. This heat of wetting can, however, be measured in an indirect way, as follows: Weighed amounts of a soil are brought to equilibrium with different humidities and consequently made to take up definite but varying amounts of moisture. Heat of wetting is then measured in every case. The difference in the heat of wetting between the dry and the moist soil is taken as that due to the initial moisture content.

EXPERIMENTAL

Sulfuric acid-water mixtures were used for controlling the humidities. The use of such mixtures for obtaining definite humidities has been discussed by Wilson (7). To obtain mixtures of definite relative humidities it is only necessary to take the densities, and then from the relation between the two, the humidity is computed. In a chemical laboratory sometimes it is easier to determine normality instead of density: a single titration with N NaOH is all that is necessary. Density and relative humidity values at various normalities are given in table 1. When plotted, these values give smooth curves from which intermediate values can be interpolated.

Three soils were chosen for this study; namely, a black cotton soil of high

base-exchange capacity containing 62 per cent clay (P.C. 13); a raw clay subsoil containing 92 per cent clay (P.C. 123); and a typical alluvial soil containing 23 per cent clay (P.C. 1112).

The soils were kept over H_2SO_4-water mixtures of different humidities in desiccators for several days until equilibrium was attained. Prior to this, they had been kept over night in a humid atmosphere, so that all soil samples lost moisture in attaining equilibrium at various humidities. This procedure is necessary, as the equilibrium moisture content at any humidity depends on whether the soil has absorbed or given up moisture (6). The curves showing the relation between the relative humidity and the moisture content for the

TABLE 1

Relation between normality, relative humidity, and density of sulfuric acid-water mixtures

SERIES NUMBER	NORMALITY	RELATIVE HUMIDITY	DENSITY 15°C./4°C.	SERIES NUMBER	NORMALITY	RELATIVE HUMIDITY	DENSITY 15°C./4°C.
	N	*per cent*			*N*	*per cent*	
1	1.0	98.7	1.033	21	11.0	50.7	1.3315
2	1.5	97.8	1.0505	22	11.5	47.5	1.3460
3	2.0	96.8	1.0680	23	12.0	44.3	1.3600
4	2.5	95.5	1.0835	24	12.5	41.4	1.3740
5	3.0	94.2	1.0970	25	13.0	38.4	1.3860
6	3.5	92.7	1.1120	26	13.5	35.7	1.3980
7	4.0	90.9	1.1280	27	14.0	33.0	1.4100
8	4.5	88.8	1.1440	28	15.0	27.75	1.4370
9	5.0	86.7	1.1575	29	16.0	22.70	1.4630
10	5.5	84.5	1.1705	30	17.0	18.0	1.4920
11	6.0	82.2	1.1860	31	18.0	13.7	1.5220
12	6.5	79.5	1.2010	32	19.0	10.4	1.5470
13	7.0	76.4	1.2200	33	20.0	7.6	1.5740
14	7.5	74.1	1.2320	34	21.0	5.1	1.6000
15	8.0	71.3	1.2470	35	22.0	3.4	1.6260
16	8.5	68.0	1.2660	36	23.0	2.25	1.6540
17	9.0	64.8	1.2760	37	24.0	1.55	1.6745
18	9.5	61.0	1.2920	38	25.0	0.95	1.6970
19	10.0	57.4	1.3060	39	26.0	0.55	1.7210
20	10.5	54.0	1.3200	40	27.0	0.35	1.7440

three soils are given in figure 1. It is interesting to note that soil P.C. 123, although it has a higher clay content than P.C. 13, actually absorbs less moisture at all humidities below 90 per cent. The change-over in the curve at higher humidities is due to the fact that soil P.C. 123 has a higher dispersion coefficient, which is associated with a high moisture absorption, between 90 and 100 per cent humidity (5).

Heat of wetting was measured in a Dewar cylinder with a Beckman thermometer. Approximately 5 gm. of soil was added to 50 cc. of water. Heat of wetting in calories per gram (Q) of soil was calculated from the following formula:

$$Q = 1/p \{(p + q)\, s + w\}(t_1 - t_0)$$

where p is the weight of dry soil, q the weight of the liquid, s the specific heat of the soil suspension, w the water value of the calorimeter, thermometer,

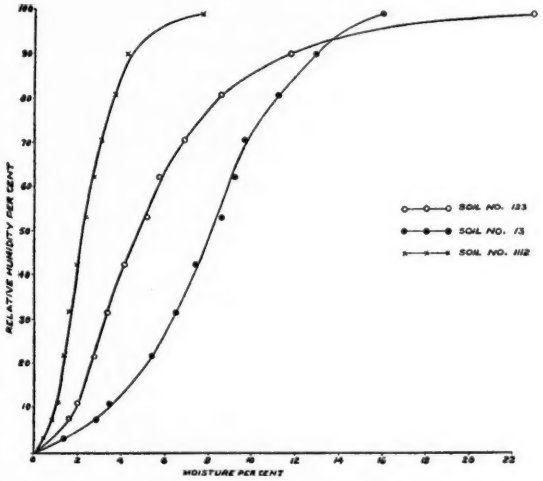


FIG. 1. RELATION BETWEEN RELATIVE HUMIDITY AND MOISTURE PERCENTAGE OF SOILS

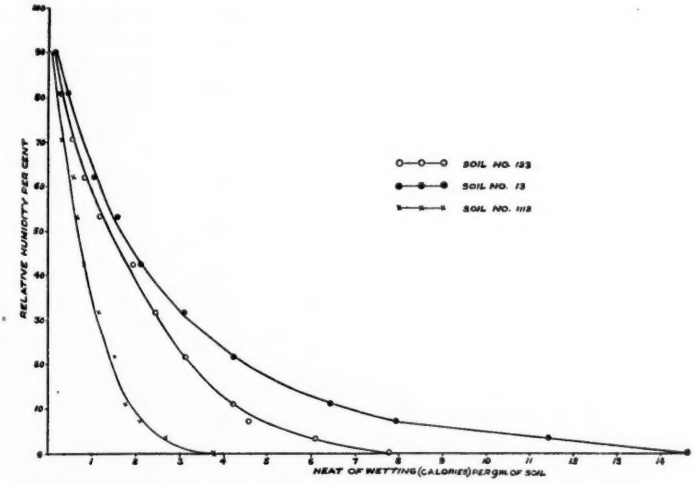


FIG. 2. RELATION BETWEEN HEAT OF WETTING AND RELATIVE HUMIDITY OF SOIL

stirrer, etc., t_0 the initial temperature of soil and the liquid, and t_1 the final temperature of the suspension.

The results are plotted in figure 2. Above 90 per cent humidity, scarcely

any heat is developed, though the soil continues to absorb moisture. It must be admitted that the moisture-humidity curves show no sharp break at 90 per cent humidity, but they definitely tend to become more and more asymptotic beyond this point. It is interesting to note that the relative positions of

TABLE 2

Relation of heat of wetting, moisture content, and clay content of various soils

SOIL NUMBER	MOISTURE	CLAY	HEAT OF WETTING	SOIL NUMBER	MOISTURE	CLAY	HEAT OF WETTING
	per cent	*per cent*	*cal./gm.*		*per cent*	*per cent*	*cal./gm.*
1	1.480	21.32	1.495	116	1.375	29.16	1.908
6	2.095	32.80	3.089	119	9.084	57.76	7.822
7	1.650	28.92	2.329	121	3.285	30.20	37.15
62	2.792	25.32	9.352	123	11.020	94.96	11.21
63	2.817	21.24	2.773	125	2.569	22.32	2.683
64	1.804	19.20	2.522	127	3.963	31.68	4.077
65	2.800	20.28	1.999	128	4.415	32.28	4.691
66	2.991	17.68	2.017	129	3.619	39.36	4.065
67	2.979	16.68	2.694	130	3.640	42.04	4.489
68	2.674	17.30	3.031	131	3.503	43.88	4.437
69	2.594	17.92	2.518	133	1.260	17.48	0.828
70	2.124	29.24	2.852	135	1.051	15.28	1.158
71	2.404	34.60	3.007	136	1.473	16.40	2.159
72	3.567	45.08	3.182	137	1.21	19.28	1.16
73	4.836	29.72	4.910	138	1.358	19.00	1.825
74	3.072	24.80	4.710	141	13.92	62.52	15.193
79	3.696	25.96	4.213	142	6.906	63.80	9.684
82	6.452	25.32	4.068	144	2.024	26.80	2.337
83	2.704	25.40	6.688	146	8.104	75.44	14.460
88	2.55	23.08	3.191	147	10.52	75.36	15.30
91	2.699	29.80	2.684	148	10.276	71.20	15.810
92	2.561	29.48	2.687	149	7.844	65.94	9.858
94	3.021	30.08	3.019	152	3.691	21.68	3.39
97	2.796	28.64	3.537	153	1.846	22.00	2.50
98	2.308	21.40	2.689	154	2.543	27.56	3.02
101	1.754	25.48	2.175	155	2.535	28.44	3.523
107	4.376	40.12	1.998	163	2.007	20.04	2.92
108	2.813	35.44	4.604	166	5.798	68.64	6.565
109	2.388	27.96	2.858	167	1.552	23.76	2.327
110	1.239	19.84	1.674	168	1.104	10.40	1.821
111	1.783	15.76	1.657	169	0.843	10.80	1.156
112	2.237	17.56	1.665	171	4.245	33.46	5.537
114	3.191	23.44	1.672				
115	2.455	20.00	1.687				

the heat of wetting curves are the same as those of the moisture-humidity curves, and that there is probably a significant correlation, therefore, between heat of wetting and hygroscopicity. This was confirmed by the observation that for a number of soils the heat of wetting in calories was found to be the same as the air-dry moisture in percentage (table 2). All the soils had been

treated with 0.05 *N* HCl and then air dried some time before these measurements. It must be admitted that air-dry moisture is not a fundamental constant, as it obviously depends on the humidity of the atmosphere prevailing at the time of drying the soil, but it does seem that laboratory conditions under which the air-dry samples are kept have a tendency to acquire a certain degree of uniformity. The correlation coefficient between heat of wetting and air-dry moisture was found to be 0.946, and that between heat of wetting and clay content, 0.887. Both these values are highly significant. The partial correlations, which isolate the effect of moisture and clay (independently of their mutual relations) on heat of wetting, were determined as 0.382 and 0.760. These indicate that heat of wetting is likely to be much more accurately linked with hygroscopicity than with clay.

Effect of exchangeable bases on heat of wetting

Heat of wetting with single-base soils was studied by Pate (3), who concluded that soil with a monovalent base gave a lower heat of wetting than that of soil with a divalent base. These conclusions were confirmed by Puri (4). The heat of wetting of single-base soils in equilibrium with different humidities has not been measured heretofore. Single-base soils were prepared from soil P.C. 13 by 0.05 *N* HCl treatment followed by neutralization with various hydroxides. The moisture-humidity curves of the soils are given in figure 3, and the heat of wetting curves in figure 4. The lower heat of soils with monovalent exchangeable bases, as compared with divalent, is rather extraordinary. In view of the high hydration of Li- and Na-soils, one would have expected that the heat of hydration would be added to the heat of wetting, and that a higher value would be obtained. A possible explanation of this difference might lie in the fact that monovalent bases are ionized to a greater extent on the soil surface and the negative heat of ionization lowers correspondingly the heat of wetting.

Heat of wetting with organic liquids

If the micropore hypothesis that offers a purely physical explanation of the mechanism of moisture absorption is correct, we should expect all other liquids that can wet the soil, and consequently enter into the micropores, to show a heat of wetting not far removed from that with water. On the other hand if the micropores are partly filled with water, the heat of wetting of the moist soil with organic liquids can be modified in two ways, as follows:

The heat of dilution of water, contained in the soil, with the organic liquid, if positive, will be added to, and, if negative, will be subtracted from, the heat of wetting of the moist soil.

The radius of curvature of the capillary water will be affected by the change in the surface tension of water in air, being replaced by the interfacial tension of the organic liquid and water. In other words, a liquid with a low interfacial tension would reduce the radius of curvature and cause the moist soil to appear wetter at a particular humidity, and thus lower the heat of wetting correspondingly.

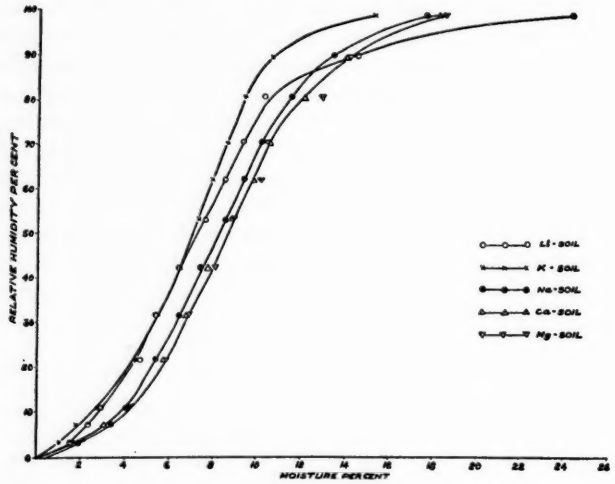


FIG. 3. RELATION BETWEEN RELATIVE HUMIDITY AND MOISTURE PERCENTAGE SOILS OF CONTAINING DIFFERENT SINGLE BASES

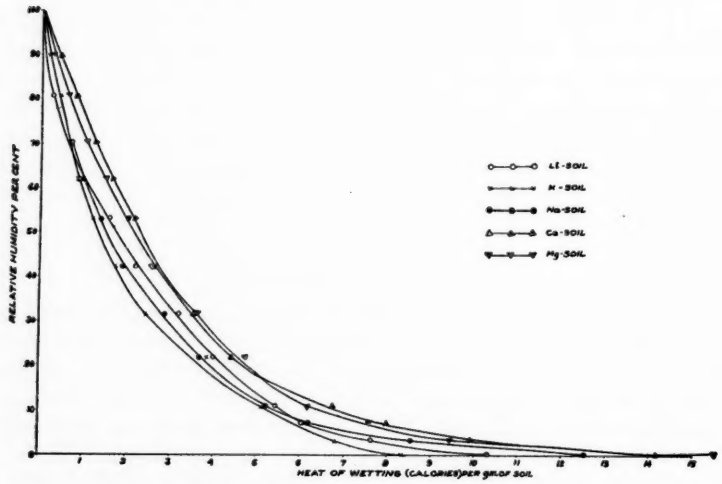


FIG. 4. RELATION BETWEEN RELATIVE HUMIDITY AND HEAT OF WETTING SOILS OF CONTAINING DIFFERENT SINGLE BASES

We can test both these possibilities by determining the heat of wetting of soils in equilibrium with different humidities, using organic liquids of known

interfacial tension and heat of dilution with water. The following liquids were chosen:

	SURFACE TENSION	INTERFACIAL TENSION
	dynes	*dynes*
Benzene	28.86	35.00
Aniline	42.58	5.77
Carbon tetrachloride	26.66	45.0

The heat of wetting of soil P.C. 13 kept at various humidities and consequently containing different amounts of moisture was studied. The curves showing the relation between heat of wetting and the relative humidity with

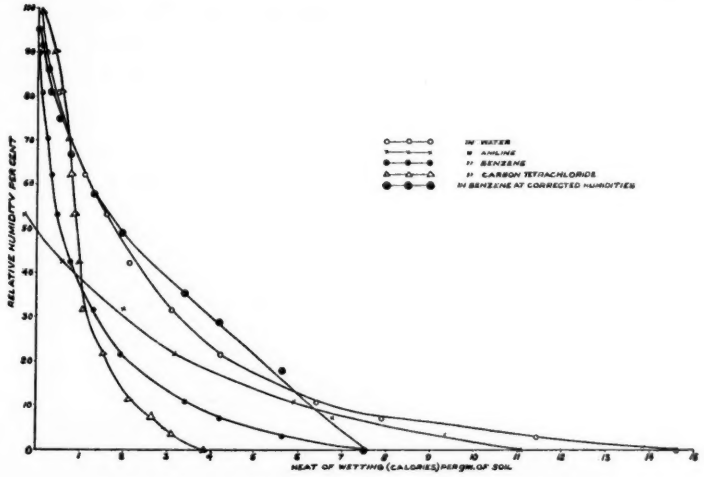


FIG. 5. HEAT OF WETTING OF SOIL IN DIFFERENT LIQUIDS

which the soil was in equilibrium are shown in figure 5. It will be seen that the heat of wetting is lowered at all humidities in the case of organic liquids. From the lowering of the freezing point of benzene by moist soils in equilibrium with different humidities, Crowther and Puri (2) concluded that the abnormal behavior of soils as compared to H_2SO_4-water mixtures and other hygroscopic substances was due to the fact that water-benzene interfacial tension is lower than water-air surface tension and that, therefore, a corresponding change in the radius of curvature of the water held in the minute capillaries is possible. It was shown that

$$p''/p = [p'/p]^{0.478}$$

where p is the vapor pressure at a plane surface, p' the vapor pressure at the curved surface, and p'' the aqueous vapor pressure at a water-benzene surface for the same capillary.

Thus the soil in equilibrium with a particular humidity appeared wetter when brought in contact with benzene, as if it were in equilibrium with a higher humidity. This conclusion seemed to conform with the data on the heat of wetting with benzene; consequently, if we calculate the relative humidity of the soil in contact with benzene, by the formula given above, and interpolate the heat of wetting corresponding to the corrected value from the curve, the corrected values fall on the heat of wetting curve for water. These values are shown in figure 5. If, however, we calculate similar values for aniline or carbon tetrachloride, by substituting the interfacial tensions of these compounds, we do get a very poor fit. Two possibilities present themselves: either some disturbing factors, which are absent in the case of benzene, are operating in the case of aniline and carbon tetrachloride; or the real cause of the difference lies in some factor other than the interfacial tension, and in the case of benzene its numerical value happens to be of the same order as the interfacial tension. As a matter of fact, the heats of wetting with the various liquids follow the order of their surface tensions and not the interfacial tensions.

TABLE 3

Relation between heat of wetting of soil (P.C. 13), methyl alcohol, and sodium oleate solutions

DESCRIPTION	HEAT OF WETTING			
	In methyl alcohol	In 0.01 *N* sodium oleate solution	In 0.2 *N* sodium oleate solution	In water
	cal./gm.	*cal./gm.*	*cal./gm.*	*cal./gm.*
Soil dried over conc. H_2SO_4.....	15.65	16.55	19.63	14.61
Soil kept over H_2SO_4-H_2O mixture of 50 per cent relative humidity.	1.50	1.02	2.21	1.65

In order to see if surface tension plays an important part in determining the magnitude of the heat of wetting, measurements were carried out with methyl alcohol and water solutions of sodium oleate. The former was chosen for the presence of the OH group and the latter for its low surface tension. The results are given in table 3. It is seen that both methyl alcohol and sodium oleate solutions give heats of wetting which are of the same order as that in pure water, in spite of the fact that surface tension is very much lower in these solutions. The results lead to the conclusion that surface tension, as such, plays very little part in determining the magnitude of the heat of wetting.

SUMMARY

Heat of wetting values of single-base soils in equilibrium with different relative humidities were measured. The results indicate that soils in equilibrium with relative humidity over 90 per cent show scarcely any heat of wetting.

There is a significant correlation between the heat of wetting and the air-dry moisture content of soils.

REFERENCES

(1) ANDERSON, M. S. 1924 The heat of wetting of soil colloids. *Jour. Agr. Res.* 28: 927–936.

(2) CROWTHER, E. M., AND PURI, A. N. 1924 The indirect measurement of the aqueous vapour-pressure of capillary systems by the freezing-point depression of benzene. *Proc. Roy. Soc.* [*London*] *Ser.* A 106: 232–242.

(3) PATE, W. W. 1925 The influence of the amount and nature of the replaceable base upon the heat of wetting of soils and soil colloids. *Soil Sci:* 20: 329–335.

(4) PURI, A. N. 1930 Studies in soil colloids: IV. Method of estimating soil colloids. *Mem. Dept. Agr. India, Chem. Ser.* 11: 119–131.

(5) PURI, A. N. 1932 A new method of determining clay content of soils by moisture absorption at 70 per cent humidity. *Soil Sci.* 33: 405–411.

(6) PURI, A. N., CROWTHER, E. M., AND KEEN, B. A. 1925 The relation between the vapour pressure and water content of soils. *Jour. Agr. Sci.* 15: 68–88.

(7) WILSON, R. E. 1921 Humidity control by means of sulfuric acid solutions with critical compilation of vapor pressure data. *Jour. Indus. and Engin. Chem.* 13: 326–331.

EFFECT OF CERTAIN MINERAL ELEMENTS ON SOME MICROBIOLOGICAL ACTIVITIES IN MUCK SOILS[1]

L. M. TURK

Michigan Agricultural Experiment Station

Received for publication January 3, 1939

Most crops grown on muck soil in Michigan respond favorably to applications of phosphorus and potash. Furthermore, certain crops grown on muck soils with certain characteristics respond especially well to such materials as $CuSO_4$, NaCl, $MnSO_4$, sulfur, boron, nitrogen, and lime. Crop adaptation and crop response to fertilizer treatment on these soils are largely determined by the reaction (pH) associated with the lime content of the soils. On this basis, all the muck soils may be placed in one of three groups as follows: very strongly acid muck (lime content low), with pH 4.5 or less; strongly acid to neutral muck (lime content sufficient), pH 4.6 to 7.0; alkaline muck (lime content very high), pH above 7.0.

The benefits derived from $CuSO_4$ are largely confined to the acid mucks. Nearly all crops are benefited from its application on the very strongly acid mucks, and many crops will show a benefit on the mediumly acid mucks. Some crops will show a response on the slightly acid and slightly alkaline mucks. The specific reasons for the beneficial influence of $CuSO_4$ on these plants are not thoroughly understood.

Sodium chloride has given favorable response on both the alkaline and the acid mucks to such crops as mangels, sugar beets, table beets, swiss chard, and celery. The benefits, however, are not likely to be so pronounced on the alkaline muck as on those with an acid reaction. The benefit derived from the salt appears to be due to an actual need for sodium by the plants.

Manganese sulfate, applied at the rate of 100 to 200 pounds an acre, has proved beneficial to certain muck crops such as radishes and spinach on those soils which have an alkaline reaction. There appears to be a definite deficiency of soluble manganese on such soils, but this deficiency can be corrected permanently and cheaply by the application of sulfur.

There is some evidence that the addition of boron may benefit certain crops grown on muck soil. It may help overcome the disease known as cracked stem in celery and that known as scab in table beets.

Sulfur is generally used on highly alkaline mucks as a corrective for the unproductive condition known locally as "alkali." The benefits derived from

[1] Authorized for publication as journal article 343 n.s. of the Michigan Agricultural Experiment Station. Contribution of the Soils Section.

sulfur applications are believed to be due primarily to the effect in neutralizing the "alkali," as a result of which, certain plant nutrients are made more available.

In general, all very strongly acid mucks will require an application of lime before satisfactory yields of most crops can be obtained.

The use of commercial fertilizers high in potash is necessary for the production of good crops on muck soils. Generally, nitrogen is not so important, in muck-land fertilization, as are phosphorus and potassium. All mucks require potash, and most of them require phosphate, especially when they are being heavily cropped.

In the absence of some of the aforementioned materials, the crop may be a complete failure, or the yield greatly decreased, yet a satisfactory explanation is lacking for many of the observed beneficial effects. There is the possibility that these benefits are brought about, in part, indirectly through the effect of the amendments on activities of soil microorganisms. The chemicals may induce changes in the biological flora or in its activities in the soil, which in turn determine, to a large extent, the supply of certain available plant nutrients. For example, $CuSO_4$ added to muck in the usual quantities may stimulate ammonification and nitrification, or it may increase the activities of the biological flora as a whole, thereby hastening the decomposition of the muck; either effect might account for increased crop yields. On the other hand, the addition of $CuSO_4$ may exert an inhibiting influence on the activities of certain groups of microorganisms, thereby decreasing the competition of the microorganisms for available plant nutrients.

The object of this investigation was to study the effect of adding various mineral elements to muck soils on certain of the microbiological processes. It was hoped that these results might aid in explaining observed crop response.

PLAN OF INVESTIGATION AND METHODS USED

Several methods were used for determining or indicating microbiological response. It is realized that there are certain limitations to almost any method for making a microbial soil analysis, but, by the use of several methods, it is possible to gather information of a more definite nature. The following methods of studying microbial response were used throughout this investigation: ammonification, nitrification, carbon dioxide production, and changes in numbers of bacteria and fungi.

It is generally believed that nitrifying organisms are more sensitive than ammonifiers to soil treatment because the ammonifying group of organisms includes a large number of organisms having varied characteristics. Many influences which retard nitrification may, therefore, have little or no effect on ammonification. As a general rule, ammonia does not accumulate in soils, and unless ammonia production is increased it is impossible for nitrate production to be increased, since ammonification must precede nitrification. Hence, soil treatment may retard or prevent nitrification and not ammonifica-

tion, but the latter process could not be restricted without also restricting nitrification.

Ammonia and nitrate accumulation studies were made by the tumbler method, and the soils were incubated at room temperature. The ammonia and nitrate determinations were made on the same soil extract, which was obtained by leaching the soil with 4 per cent KCl. An aliquot of the extract was made alkaline, and the ammonia was distilled into boric acid and titrated with H_2SO_4. Nitrates were determined by the reduction method. Carbon dioxide was measured, in general, by the method previously outlined.[2] Plate counts of bacteria and fungi were made according to the methods outlined by Fred and Waksman.[3] Determinations of pH were made electrometrically with the use of a glass electrode.

The effects of N, P, and K were investigated on all types of muck studied. The effects of different kinds of lime at varying rates were studied, mainly on the acid mucks. In some instances, however, lime was added to neutral and alkaline mucks to study the possible effects of overliming. Studies were made concerning the effects of applying sulfur at different rates to alkaline mucks. The possible effects of several so-called "minor elements" were also investigated. In this group of materials used may be listed the following: $CuSO_4$, NaCl, $MnSO_4$, KI, $BaCl_2$, $Al_2(SO_4)_3$, $FeSO_4$, $ZnSO_4$, H_3BO_4, and Na_2SO_4. $CuSO_4$, NaCl, and $MnSO_4$ were applied at different rates, whereas each of the other materials was applied at only one rate. In general, these minor elements were applied to the muck in addition to the regular fertilizer treatments. Most of the experiments were set up in two series: a lime series and a no-lime series.

A few studies were made of the effect of $MnSO_4$ and $CuSO_4$ on nitrification in nutrient solution cultures. Sodium nitrite was added to a nutrient solution favorable for nitrate formers, and the various flasks were inoculated with a muck infusion. The main object of this study was to determine whether $MnSO_4$ and $CuSO_4$ stimulate or retard nitrification and to determine the quantity of $CuSO_4$ necessary to prevent nitrification.

Barnyard manure has been used on muck soils with satisfactory results on some crops. In order to determine what effect manure might have in increasing the ammonifying and nitrifying capacity of soils, a manure infusion was added to several muck soils. This study was made for the purpose of determining whether or not the nitrifying capacity or the rate of nitrification is limited as a result of an insufficient number of active nitrifying organisms.

In most of the experiments dealing with ammonia and nitrate accumulation, enough tumblers for each treatment were set up to allow periodical analyses

[2] Turk, L. M. 1932 The composition of soybean plants at various growth stages as related to their rate of decomposition and use as green manure. Missouri Agr. Exp. Sta. Res. Bul. 173.

[3] Fred, E. B., and Waksman, S. A. 1928 Laboratory Manual of General Microbiology. McGraw-Hill Book Co., New York.

for 6 to 12 weeks and in some instances for 15 weeks. In each experiment, analyses were usually made at intervals of 2, 3, or 4 weeks, but, in general, only the data for the longest incubation period are reported.

Muck soils of varying degrees of acidity were used. The studies here reported were all made under laboratory conditions.

As soon as the soil samples were brought into the laboratory they were put through a 4-mesh screen to remove the coarsest material and were stored in the moist condition as they were brought in from the field. No samples were air-dried. In most instances the experiments were started immediately after the samples were brought to the laboratory. Previous to setting up the experiments, the following determinations were made on each sample of muck: ammonia nitrogen, nitrate nitrogen, moisture, and pH.

The name applied to each of the soils used in this report refers to the name of the farmer on whose farm the sample was taken, or to the name of the place (town, village, etc.) close to where the sample was taken. The names have no meaning other than to indicate the source of the various samples.

EXPERIMENTAL RESULTS

Ammonia and nitrate accumulation

The mucks used and their treatments for the studies of ammonia and nitrate accumulation are shown in tables 1 to 7. The equivalent of 25 gm. oven-dry muck was used in each tumbler for all these experiments. Lime, where used, was added in the form of $CaCO_3$ except where otherwise indicated in the tables. The 0–8–24 and the 3–8–24 fertilizers and all the other chemicals except lime and sulfur were added in solution. High-grade (c.p.) chemicals were used throughout. Potassium chloride and $CaH_4(PO_4)_2$ were used to make the 0–8–24 fertilizer and to supply the P and K in the 3–8–24 fertilizer. Ammonium sulfate was used to supply the nitrogen in the 3–8–24 fertilizer. The treatments were all made on the basis of the weight of muck. The samples were thoroughly mixed and brought up to optimum moisture at the start of the experiment and periodically throughout the incubation periods.

It will be observed that a few chemicals have been used (see tables 4 and 7) which, in general, do not increase crop growth on muck soils under field conditions.

The data assembled in tables 1 and 2 were obtained from various mucks which were given identical treatments except for the lime treatments. In some instances $Ca(OH)_2$ was used and in others $CaCO_3$. Calcium hydroxide was applied at a rate equivalent to 5 tons $CaCO_3$ to the Trowbridge muck (pH 4.11) and to the Mt. Hope muck (pH 3.4). The lime increased nitrification somewhat in the Trowbridge muck, but on the whole did not increase ammonia accumulation. In most instances the $Ca(OH)_2$ resulted in less ammonia at the end of 6 weeks than at the beginning. This was probably due to a stimulating effect on the nitrifying organisms. In other words, the

TABLE 1

Effect of various mineral elements on ammonia and nitrate accumulation in muck soils

N as mgm. per 100 gm. oven-dry muck

TREATMENT PER ACRE	INCREASE OR DECREASE OF N (AS AMMONIA AND NITRATE) DURING INCUBATION PERIOD INDICATED											
	Trowbridge muck* pH 4.11 (6 weeks)				Trowbridge muck After CO_2 production studies* (10 weeks)		Mt. Hope muck† pH 3.4 (6 weeks)				Mt. Hope muck† After CO_2 studies (10 weeks)	
	No lime		Lime‡		No lime		No lime		Lime‡		No lime	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
1. Check	−4.8	2.1	−0.9	6.0	−1.2	4.5	13.0	1.9	33.4	4.3	10.6	2.8
2. 1000 lbs. 0–8–24	−4.6	3.3	−9.9	10.8	−0.3	3.3	13.3	3.6	32.7	9.1	9.6	5.7
3. 1000 lbs. 0–8–24 50 lbs. $CuSO_4$	−5.4	3.3	−0.3	4.2	−0.9	8.4	13.6	5.3	36.4	6.0	11.9	4.7
4. 1000 lbs. 0–8–24 100 lbs. $CuSO_4$	−5.4	0.9	−9.0	10.8	−0.3	4.2	13.6	6.0	35.7	5.3	13.3	4.5
5. 1000 lbs. 0–8–24 1000 lbs. NaCl	−4.8	5.1	−0.3	5.1	0.9	8.1	13.0	11.8	36.1	4.3	11.4	5.2
6. 1000 lbs. 0–8–24 2000 lbs. NaCl	−4.8	1.5	−4.8	2.1	2.1	5.1	18.4	1.6	33.7	2.6	15.0	5.7
7. 1000 lbs. 0–8–24 2000 lbs. NaCl 100 lbs. $CuSO_4$	−5.1	1.2	−8.1	5.4	2.1	3.9	14.7	0.9	38.5	1.3	10.8	1.3
8. 5000 lbs. 3–8–24	1.2	0.3	−3.6	11.1	11.4	2.1	21.3	4.0	45.1	1.3	16.4	1.3
9. 5000 lbs. 3–8–24 100 lbs. $CuSO_4$	1.2	−0.3	2.1	2.4	11.1	1.2	21.3	0.2	43.8	0.6	18.6	0.2
10. 5000 lbs. 3–8–24 2000 lbs. NaCl	11.8	1.5	−2.7	7.2	14.7	9.3	24.4	8.4	43.4	8.4	21.3	5.0
11. 5000 lbs. 3–8–24 2000 lbs. NaCl 100 lbs. $CuSO_4$	5.7	1.5	0.0	3.6	13.5	4.5	20.0	8.7	42.4	6.4	21.5	3.5
12. 5000 lbs. 3–8–24 400 lbs. $MnSO_4$	7.5	1.5	−18.9	15.3	13.5	3.0	20.6	0.9	43.4	2.6	22.0	1.1

* 11.4 and 1.8 mgm. ammonia and nitrate nitrogen respectively per 100 gm. untreated muck at start of experiment.

† 15.6 and 1.8 mgm. ammonia and nitrate nitrogen respectively per 100 gm. untreated muck at start of experiment.

‡ $Ca(OH)_2$ equivalent to 5 tons $CaCO_3$.

nitrifiers were stimulated to a greater extent than the ammonifiers. With the Mt. Hope muck, however, the results were just the reverse. The quantities of ammonia were greatly increased during the 6 weeks' incubation period,

TABLE 2

Effect of various mineral elements on ammonia and nitrate accumulation in muck soils

N as mgm. per 100 gm. oven-dry muck

TREATMENT PER ACRE*	INCREASE OR DECREASE OF N (AS AMMONIA OR NITRATE) DURING INCUBATION PERIOD INDICATED											
	Crane Muck† pH 4.28 (12 weeks)				College Muck‡ pH 7.0 (12 weeks)				College Muck‡ After CO_2 Production Studies (8 weeks)			
	No Lime		Lime		No Lime		Lime		No Lime		Lime	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
1. Check	3.2	12.8	2.4	53.6	−2.5	14.9	−2.1	28.7	−1.5	12.8	−3.3	24.8
2. 1000 lbs. 0-8-24	8.8	13.4	2.0	51.8	−2.5	19.4	−2.2	31.4	−1.8	9.2	−2.7	26.3
3. 1000 lbs. 0-8-24 50 lbs. $CuSO_4$	6.8	11.0	1.2	49.0	−2.4	19.1	−2.2	34.1	−1.8	10.7	−2.7	26.9
4. 1000 lbs. 0-8-24 100 lbs. $CuSO_4$	11.0	5.4	2.2	46.4	−2.7	18.2	−2.2	31.7	−2.2	11.9	−2.7	26.0
5. 1000 lbs. 0-8-24 1000 lbs. NaCl	16.4	8.4	5.2	38.2	−2.4	16.7	−2.1	27.8	−1.8	12.2	−2.4	23.3
6. 1000 lbs. 0-8-24 2000 lbs. NaCl	13.8	1.2	3.0	41.0	−2.5	14.0	−2.4	27.8	−2.1	11.6	−2.4	24.5
7. 1000 lbs. 0-8-24 2000 lbs. NaCl 100 lbs. $CuSO_4$	12.4	0.6	4.8	37.6	−2.7	14.3	−1.9	26.6	−1.5	10.7	−2.7	23.0
8. 5000 lbs. 3-8-24	12.8	6.6	−31.0	67.0	−16.4	33.2	−15.8	39.5	−15.8	27.1	−16.4	35.9
9. 5000 lbs. 3-8-24 100 lbs. $CuSO_4$	18.0	4.2	−33.2	66.8	−15.8	29.9	−15.9	39.8	−16.4	25.7	−16.4	37.4
10. 5000 lbs. 3-8-24 2000 lbs. NaCl	19.0	7.4	−32.8	57.2	−16.1	32.6	−15.9	39.5	−16.4	26.9	−16.1	32.9
11. 5000 lbs. 3-8-24 2000 lbs. NaCl 100 lbs. $CuSO_4$	14.6	4.2	−33.2	63.6	−15.8	34.4	−15.5	44.0	−16.5	31.1	−14.9	35.9
12. 5000 lbs. 3-8-24 400 lbs. $MnSO_4$	16.2	9.4	−31.4	69.2	−16.1	32.3	−15.6	38.3	−16.1	26.9	−16.1	32.6

* Treatment 12 for Crane Muck was 3500 lbs. $BaCl_2$ instead of 400 lbs. $MnSO_4$.

† 2.0 and 2.4 mgm. ammonia and nitrate N respectively per 100 gm. untreated muck at start of experiment.

‡ 3.3 and 15.7 mgm. ammonia and nitrate N respectively per 100 gm. untreated muck at start of experiment.

whereas the quantities of nitrates were only slightly changed. In some instances there were small increases and in others small decreases. It would appear that $Ca(OH)_2$ was applied in sufficient quantities to the Mt. Hope

muck to be toxic to the nitrifying organisms. For some unexplainable reason, however, $CaCO_3$ did not increase the rate of nitrification in this muck (table 3). The experiment was repeated with the same results. This was the only acid soil of the group studied in which the nitrifying organisms failed to respond favorably to additions of $CaCO_3$. The nitrifying capacity of the Crane muck, pH 4.28 (table 2), and of the Douglas peat, pH 3.4 (table 3), was decidedly increased by liming (with $CaCO_3$), and that of the College muck (table 2), although neutral in reaction at the start, was increased to a considerable extent.

TABLE 3

Effect of various mineral elements on ammonia and nitrate accumulation in muck soils

N as mgm. per 100 gm. oven-dry muck

TREATMENT PER ACRE*	INCREASE OR DECREASE OF N (AS AMMONIA OR NITRATE) DURING INCUBATION PERIOD INDICATED									
	Mt. Hope Muck† pH 3.4 (3 weeks)				College Muck‡ pH 8.0 (15 weeks)		Douglas Peat‖ pH 3.4 (6 weeks)			
	No Lime		Lime		No Lime		No Lime		Lime	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
1. Check	−5.1	19.2	−6.9	17.9	−0.2	19.0	−3.8	7.6	−2.7	29.5
2. 100 lbs. $CuSO_4$	−4.8	18.3	−6.7	21.0	−0.2	23.6	−3.2	7.6	−3.4	33.7
3. 100 lbs. KI	−4.8	18.6	−6.0	21.0	0.6	18.6	0.8	6.4	5.5	20.8
4. 1000 lbs. $BaCl_2$	−4.8	21.6	−6.3	19.5	−0.2	18.2	3.0	2.6	1.0	25.3
5. 100 lbs. $MnSO_4$	−4.5	29.4	−6.3	17.7	0.7	21.0	−2.4	6.4	4.8	26.7
6. 100 lbs. $Al_2(SO_4)_3$	−4.6	21.6	−6.4	18.9	0.4	18.0	−2.6	6.4	−1.5	29.5
7. 100 lbs. $FeSO_4$	−4.8	24.3	−6.4	20.4	0.4	22.8	−3.0	8.6	−0.4	33.0
8. 100 lbs. $ZnSO_4$	−4.5	25.8	−6.6	22.8	−0.5	19.6	−2.8	7.4	1.1	25.3
9. 100 lbs. Boric Acid	−4.6	14.7	−6.0	21.0	0.1	17.5	−2.0	6.6	−3.2	30.2
10. 100 lbs. Na_2SO_4	−4.8	14.4	−6.1	24.6	−0.1	20.9	−1.8	8.0	−4.8	36.5
11. 1000 lbs. NaCl	−4.6	12.9	−6.1	24.3	−0.2	16.2	4.2	1.2	−5.9	29.9
12. 2000 lbs. Sulfur	−4.8	14.4	−6.0	19.8	0.1	29.7	−4.0	7.8	3.6	33.7

* Treatment 12 for Douglas peat was 100 lbs. molybdic acid per acre instead of 2000 lbs. sulfur. All chemicals, except sulfur and lime, added in solution.

† 6.3 and 10.2 mgm. ammonia and nitrate nitrogen respectively per 100 gm. untreated muck at start of experiment.

‡ 1.5 and 22.8 mgm. ammonia and nitrate nitrogen respectively per 100 gm. untreated muck at start of experiment.

‖ 9.2 and 7.6 mgm. ammonia and nitrate nitrogen respectively per 100 gm. untreated muck at start of experiment.

In five cases out of seven (tables 1 and 2) the 0–8–24 fertilizer, in the no-lime series, increased the quantities of nitrates in comparison to the check, and the same fertilizer, in the limed series, gave increases in four out of five cases. In most instances the increases due to the 0–8–24 were not great.

In all but one of the mucks listed in tables 1 and 2, the 5000-pound-per-acre application of 3–8–24 fertilizer, in the limed series, significantly increased the quantities of nitrates that accumulated. (This increase was due largely to

the applied nitrogen.) The one exception was the Mt. Hope muck to which a heavy application of $Ca(OH)_2$ was made. In the unlimed soils, the 3–8–24 fertilizer resulted in increased nitrate accumulation in only about one-half the cases. It is possible that a longer incubation period would have modified the results.

The effects of $CuSO_4$ (both the 50- and 100-pound-per-acre applications) were not uniformly positive in affecting nitrate accumulation in the mucks studied (tables 1 and 2). In comparing the 0–8–24 treatments with those that received $CuSO_4$ in addition, it is observed that $CuSO_4$ did not consistently affect nitrification. There are as many instances showing a decrease in nitrates as there are showing an increase. This is true on both the limed and the unlimed series. In comparing the 100-pound treatment of $CuSO_4$ plus the 3–8–24 fertilizer with the 3–8–24 fertilizer alone, it is found that $CuSO_4$ resulted in decreased nitrate accumulation in every instance on the no-lime series and in over 50 per cent of the cases on the limed series. There were no consistent differences in the production of nitrates between treatment 10, consisting of 3–8–24 fertilizer and 2000 pounds NaCl, and treatment 11, consisting of 100 pounds per acre of $CuSO_4$ in addition to the 3–8–24 and NaCl. The number of instances showing an increase in nitrates about equalled that showing a decrease. This was true on both the limed and the unlimed soils. Copper sulfate (100 pounds per acre) in addition to 2000 pounds NaCl on the mucks receiving 0–8–24 fertilizer (comparing treatments 6 and 7) caused a decrease in the accumulation of nitrates in 10 out of 12 cases (including both limed and unlimed soils). In all the comparisons (about 60) involving $CuSO_4$, only about one-third of the cases resulted in increased nitrate accumulation and about two-thirds resulted in decreased nitrate accumulation.

In summarizing the results of the effect of NaCl on ammonification and nitrification, a comparison was made of the following treatments: 5 with 2, 6 with 2, and 10 with 8 (tables 1 and 2). Nitrate accumulation was less in 21 of 36 instances, and ammonia accumulation was greater in 25 of 36 instances, where NaCl was added at the rate of 1000 or 2000 pounds per acre to limed as well as unlimed soils. A somewhat different picture of the effect of NaCl is obtained by comparing the lime with the no-lime series of these treatments. Nitrate accumulation was higher in only 1 of 15 instances on the lime series and in 11 of 21 where no lime was applied. Ammonia accumulation was greater in 11 of 15 cases on the lime series, and in 14 of 21 on the no-lime series. These results indicate that, in general, the application of NaCl brought about increases in ammonia nitrogen by retarding the activities of the nitrifying organisms, particularly in the mucks that were limed.

To determine the combined effect of NaCl and $CuSO_4$ on nitrification, the following comparisons are made: treatment 7 with 2, and treatment 11 with 8 (tables 1 and 2). In the first comparison (7 and 2) it is found that a combination of NaCl and $CuSO_4$ in addition to the 0–8–24 fertilizer decreased nitrate accumulation in every instance on the limed soils and in five out of seven in-

stances on the unlimed soils. On the other hand, NaCl and $CuSO_4$ used with the 3–8–24 fertilizer resulted in greater accumulation of nitrates on the unlimed soil in six out of seven cases in comparison to the 3–8–24 fertilizer alone, but no consistent differences were obtained on the limed soils. These differences are probably due to the effect of NaCl rather than $CuSO_4$, as indicated by the results obtained with the materials used individually.

The quantities of nitrates in the mucks receiving treatment 7 (tables 1 and 2), consisting of 2000 pounds of NaCl and 100 pounds $CuSO_4$ per acre in addition to the 0–8–24 fertilizer, were lower in every instance on both the limed and the unlimed soils than were those in the checks.

Treatment 11 (a combination of 3–8–24, NaCl, and $CuSO_4$), in comparison with the check, brought about increased quantities of nitrates in most cases. That would be expected because of the addition of considerable soluble nitrogen in the form of $(NH_4)_2SO_4$. In general, the same effect is noted in a comparison of the results of treatments 11 and 7.

The data for treatments 12 and 8 (tables 1 and 2), give no evidence that $MnSO_4$ either retarded or hastened ammonification or nitrification on either the limed or the unlimed mucks. In some instances increases were obtained and in others decreases, but, in the main, the differences were not significant.

With the exception of $FeSO_4$, sulfur on alkaline soils, and lime on the Douglas peat, the treatments indicated in table 3 were generally ineffective in increasing nitrate accumulation. The $FeSO_4$ stimulated nitrification in all three soils on both the limed and the unlimed series. There were no lime treatments on the College muck. The addition of sulfur, however, increased nitrification significantly in the College muck (pH 8.0) and to some extent in the Mt. Hope muck which was limed, but no response was obtained from sulfur on the unlimed acid soils. Lime, applied to the Douglas peat, was decidedly effective in increasing nitrification, regardless of whether it was used alone or in combination with any of the minor elements applied.

As applications of sulfur at the rate of 2000 pounds per acre stimulated nitrification in the alkaline mucks and in those that were heavily limed, an experiment was set up to determine the effects of increasing quantities of sulfur on College muck (pH 7.3). The treatments made in this experiment and the results obtained are shown in table 4. An application of 0.2 gm. of sulfur to 25 gm. muck represents a 4000-pound-per-acre application (assuming that an acre 7 inches of the muck weighs 500,000 pounds). Nitrate accumulation decreased as the sulfur additions increased. The decrease was not appreciable, however, until more than 8000 pounds of sulfur per acre was added, according to the data obtained at the end of the 7-month incubation period. Below pH 5.5, nitrate accumulation was greatly decreased. The ammonifying organisms were not so adversely affected as were the nitrifiers. The greatest accumulation of ammonia occurred with the heaviest application of sulfur. This accumulation is due, no doubt, to the relative inactivity of the nitrifiers, which is due, in turn, to the intense acidity produced.

Since lime (at the rate of 5 tons per acre) was very effective in stimulating nitrate production in the Douglas peat (table 3), an experiment was run to study the effect of increasing quantities of lime. The applications of $CaCO_3$ varied from 2 to 20 tons per acre. The peat was incubated at room temperature for 5 weeks prior to the making of the ammonia and nitrate determinations.

TABLE 4

Effect of sulfur on ammonia and nitrate accumulation in College muck (pH 7.3)

N as mgm. per 100 gm. oven-dry muck

SULFUR TREATMENT*	N (AS AMMONIA OR NITRATE)				pH VALUES END 6 MONTHS
	End 6 months		End 7 months		
	NH_3	NO_3	NH_3	NO_3	
gm.					
Check	1.1	37.8	4.0	44.3	7.3
0.2	1.8	33.0	4.0	40.5	6.0
0.4	1.6	20.2	3.0	39.4	5.5
0.6	11.2	19.7	5.9	25.0	4.0
0.8	19.8	10.9	18.9	10.2	3.0
1.0	18.9	7.2	27.2	6.1	2.5

* Quantities of sulfur added to 25 gm. muck.

TABLE 5

Effect of lime on ammonia and nitrate accumulation in Douglas peat (pH 3.4)*

N as mgm. per 100 gm. oven-dry peat

LIME TREATMENT†	INCREASE OF N (AS AMMONIA OR NITRATE) DURING INCUBATION PERIOD OF 5 WEEKS		LIME TREATMENT	INCREASE OF N (AS AMMONIA OR NITRATE) DURING INCUBATION PERIOD OF 5 WEEKS	
	NH_3	NO_3		NH_3	NO_3
gm.			gm.		
Check	31.4	8.4	1.2	51.2	25.0
0.2	30.1	17.9	1.4	45.2	27.9
0.4	31.2	15.0	1.6	31.8	41.7
0.6	36.7	11.1	1.8	17.0	54.3
0.8	42.3	16.4	2.0	11.5	59.2
1.0	50.3	10.8			

* 9.2 and 7.6 mgm. ammonia and nitrate nitrogen respectively per 100 gm. oven-dry peat at start of experiment.

† Quantities of $CaCO_3$ added to 25 gm. peat.

The results obtained at that time are shown in table 5. The greatest accumulation of nitrates occurred with the heaviest lime application (20 tons per acre). From the results of this experiment it is not known whether or not the maximum rate of nitrification was attained, but under few field conditions would it ever be practical to apply more than 20 tons of lime per acre. This is a very acid peat, and although small applications of lime increased nitrate ac-

cumulation, no appreciable increases were obtained during the 5-week period except with treatments of more than 10 tons of lime per acre. These results indicate the expense involved in attempting to make such a soil favorable for rapid nitrification and good crop growth under field conditions. Unfortunately, pH determinations were not made prior to the ammonia and nitrate determinations.

The nitrifying power of some of the mucks, as reported in tables 1, 2, and 3, was very low even after the application of lime and the correction for certain other possible limiting factors. In order to test for the absence of active nitrifiers, an experiment was set up to study the effect of adding a manure

TABLE 6

Effect of manure infusion on ammonia and nitrate accumulation in various muck soils

N as mgm. per 100 gm. oven-dry muck

MUCK* AND TREATMENT	INCREASE OR DECREASE OF N (AS AMMONIA OR NITRATE) DURING INCUBATION PERIOD OF 18 WEEKS	
	NH_3	NO_3
College muck (pH 8.0)		
No manure	−0.1	11.7
Manure	−0.1	14.4
Crane muck (pH 4.28)		
No manure	20.2	4.1
Manure	23.1	3.6
College muck (pH 7.0)		
No manure	1.3	28.1
Manure	1.3	27.7
Trowbridge muck (pH 4.11)		
No manure	24.2	3.2
Manure	25.5	4.4

* See footnotes of tables 1, 2, and 3 for quantities of ammonia and nitrate nitrogen at beginning of experiment for these soils.

infusion on the accumulation of nitrates. If the nitrifying capacity of the mucks was low because of the absence of active nitrifying organisms, the capacity should be increased by adding the organisms from an infusion of fresh horse manure. The results of such an experiment are shown in table 6. There is no indication of an appreciable benefit in any case. The manure infusion contained an abundance of nitrifying organisms as was demonstrated in nitrification studies in solution cultures. The results of an experiment comparing the effect of adding a manure infusion alone and with several chemical compounds on ammonification and nitrification in College muck are reported in table 7. In only two instances did the manure infusion give increases in nitrate accumulation above the check, and these increases, obtained where the manure infusion was used with KI and with $MnSO_4$, were

TABLE 7

Effect of manure infusion and various mineral elements on ammonia and nitrate accumulation in College muck (pH 8.0)*

N as mgm. per 100 gm. oven-dry muck

TREATMENT PER ACRE	INCREASE OR DECREASE OF N (AS AMMONIA OR NITRATE) DURING INCUBATION PERIOD OF 5 WEEKS	
	NH_3	NO_3
1. Check		
No manure	−1.3	5.4
Manure	−0.9	0.6
2. 100 lbs. $CuSO_4$		
No manure	−1.2	4.8
Manure	−0.9	3.0
3. 100 lbs. KI		
No manure	−0.9	3.0
Manure	−0.6	7.2
4. 1000 lbs. $BaCl_2$		
No manure	−0.9	6.0
Manure	−0.9	3.9
5. 100 lbs. $MnSO_4$		
No manure	−0.6	0.9
Manure	−0.7	7.2
6. 100 lbs. $Al_2(SO_4)_3$		
No manure	−1.2	−6.6
Manure	−0.7	1.8
7. 100 lbs. $FeSO_4$		
No manure	−1.2	4.8
Manure	−0.9	1.8
8. 100 lbs. $ZnSO_4$		
No manure	−0.9	2.4
Manure	−0.9	−0.3
9. 100 lbs. Boric acid		
No manure	−0.7	3.3
Manure	−0.9	−1.5
10. 100 lbs. Na_2SO_4		
No manure	−0.9	−3.6
Manure	−0.9	−9.9
11. 1000 lbs. NaCl		
No manure	−1.0	−4.2
Manure	−0.9	−11.7
12. 100 lbs. Molybdic acid		
No manure	−1.2	8.7
Manure	−0.9	−2.4

* The muck contained 1.5 and 22.8 mgm. ammonia and nitrate nitrogen respectively per 100 gm. oven-dry muck at start of experiment.

not significant. Slight increases in nitrate accumulation were obtained with $CaCl_2$ and molybdic acid when added alone. None of the treatments were effective in increasing significantly nitrate accumulation above the untreated

soil. In fact, some of the treatments brought about decided decreases; this was particularly true with the application of Al_2SO_4, Na_2SO_4, and NaCl.

An experiment was set up to determine what effect $MnSO_4$ and $CuSO_4$ would have in stimulating or retarding nitrification in nutrient solution cultures and to determine the quantity of $CuSO_4$ required to prevent nitrification. The nutrient solution used for this experiment was prepared by adding 3.0 gm. K_2HPO_4, 0.6 gm. $MgSO_4$, 3.0 gm. NaCl, and 3 drops of $FeCl_3$ (10 per cent solution) to 3000 cc. distilled water. To each of a number of flasks containing 50 cc. of this solution was added 10 mgm. of nitrogen as $NaNO_2$. All flasks, except those of one series, were inoculated with 1 cc. of a College muck suspension prepared by adding 200 cc. of water to 50 gm. of muck.

The data in table 8 show that almost complete nitrification of the nitrite nitrogen had occurred by March 5 with treatments 2 and 3. The data ob-

TABLE 8

*Nitrification studies in nutrient solutions**

TREATMENT	N AS NITRATE PER FLASK†	
	March 5	March 8
	mgm.	*mgm.*
1. No inoculation	0.8	0.9
2. Inoculation	9.1	8.4
3. Inoculation + 0.0128 gm. $MnSO_4$	9.5	7.8
4. Inoculation + 0.001 gm. $CuSO_4$	8.6	9.2
5. Inoculation + 0.005 gm. $CuSO_4$	6.7	6.8
6. Inoculation + 0.01 gm. $CuSO_4$	1.6	1.6
7. Inoculation + 0.001 gm. $CuSO_4$ + 0.5 gm. $CaCO_3$		10.0‡
8. Inoculation + 0.01 gm. $CuSO_4$ + 0.5 gm. $CaCO_3$		2.2‡

* Experiment set up January 26, 1937.

† Average of duplicate determinations.

‡ Average of triplicate determinations.

tained March 5 indicate a slight benefit from the use of $MnSO_4$, but the difference is not great. The application of $CuSO_4$ retarded nitrification especially when more than 0.005 gm. was added per flask. Practically no nitrification took place where 0.01 gm. $CuSO_4$ was used. The data obtained on March 8 show the 0.001 gm. $CuSO_4$ treatment to be the highest in nitrate. On this date the nitrates were lower for the manganese treatment than on March 5, probably because of assimilation of the nitrate nitrogen by microorganisms. The heavier $CuSO_4$ treatments were definitely detrimental to the nitrifying organisms. That the low nitrate content with the heavy $CuSO_4$ treatments was not due to microbial assimilation was shown by the presence of large quantities of nitrite nitrogen. Nitrification occurred rapidly when $CaCO_3$ was used with 0.001 gm. $CuSO_4$ but was almost completely stopped with 0.01 gm. $CuSO_4$ (compare treatments 7 and 8).

Carbon dioxide production

In the CO_2 production studies, four different mucks were used, representing some of the same soils used in the ammonia and nitrate production experiments. The treatments given the mucks, the length of the incubation period, and the results of the experiments are shown in table 9. The quantities of CO_2 liberated were determined by collecting the gas in "ascarite" and weighing it on an analytical balance. The length of the period between CO_2 determinations varied considerably from one experiment to another, depending on the rate of CO_2 liberation. In some cases CO_2 was determined daily, and in others, weekly. After an 8-week incubation period with the various mineral treatments (as shown in table 9), 1 gm. of mannitol (in solution) was added to each sample of the Crane muck. Determinations were then made for CO_2 liberation during the next several days.

Lime ($CaCO_3$), whether added alone or in combination with some of the other treatments, greatly increased the liberation of CO_2 from the Crane muck (pH 4.28). This was particularly true prior to the addition of the mannitol. After the addition of mannitol, lime increased CO_2 liberation except with the 3–8–24 fertilizer treatments, and with the latter, lime gave increases where $CuSO_4$ was added. A comparison is made in figure 1 between the limed and unlimed checks and between the limed and unlimed 3–8–24 treatments prior to the addition of mannitol. The differences due to the $CaCO_3$ were largely brought about during the first 24 hours after its application. After the first day, the liberation of CO_2 was about the same for the limed and unlimed muck: the curves for the limed and unlimed 3–8–24 treatments are virtually parallel, as are those for the limed and unlimed checks. The differences in the quantities of CO_2 produced with the addition of lime are due largely to CO_2 arising from a decomposition of the carbonate added rather than from the organic matter of the muck. The fact that lime did not increase the rate of CO_2 liberation after the first day supports this contention. Furthermore, data from an experiment in which the three forms of lime [$CaCO_3$, $Ca(OH)_2$, and CaO] were compared on this same muck suggest that a large quantity of CO_2 is liberated from the carbonate when added to an acid soil. The limes were added in equivalent quantities (rate equivalent to 2 gm. $CaCO_3$ per 50 gm. muck), and, at the end of 3 days, 354, 123, and 128 mgm. of CO_2 had been liberated by the addition of $CaCO_3$, $Ca(OH)_2$, and CaO, respectively.

The curves in figure 1 for the data after the addition of mannitol show that lime increased CO_2 liberation, as did also the 3–8–24 fertilizer when added alone or with lime. This point is clearly illustrated by the spread in the curves as the incubation period was extended. In this connection, it is interesting to note that before the addition of mannitol the muck receiving the 3–8–24 fertilizer produced less CO_2 than did the check, but that after the addition of mannitol the muck receiving the 3–8–24 fertilizer produced considerably more CO_2 (fig. 1 and table 9).

The discrepancies in the liberation of CO_2 as a result of addition of mannitol

TABLE 9

Effect of various mineral elements on the production of carbon dioxide in muck soils

TREATMENT PER ACRE	TOTAL CO_2 PRODUCED DURING INCUBATION PERIOD INDICATED							
	Crane muck* (pH 4.28)		Crane muck* after addition of mannitol		Trowbridge muck† (pH 4.11)	College muck† (pH 7.0)		Mt. Hope muck† (pH 3.4)
	56 days No lime	56 days 10T. lime	16 days No lime	15 days Lime	20 days No lime	53 days No lime	55 days 10T. lime	38 days No lime
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1. Check	282	465	697	773	34	180	49	144
2. 1000 lbs. 0–8–24	278	465	694	784	37	184	48	108
3. 1000 lbs. 0–8–24 50 lbs. $CuSO_4$	254	515	684	768	36	178	58	135
4. 1000 lbs. 0–8–24 100 lbs. $CuSO_4$	243	455	696	777	37	173	53	128
5. 1000 lbs. 0–8–24 1000 lbs. NaCl	210	490	675	740	26	153	43	123
6. 1000 lbs. 0–8–24 2000 lbs. NaCl	231	454	686	755	39	156	45	111
7. 1000 lbs. 0–8–24 2000 lbs. NaCl 100 lbs. $CuSO_4$	214	391	690	788	25	156	49	105
8. 5000 lbs. 3–8–24	246	369	817	830	31	169	55	114
9. 5000 lbs. 3–8–24 100 lbs. $CuSO_4$	233	367	798	855	26	172	49	113
10. 5000 lbs. 3–8–24 2000 lbs. NaCl	222	385	820	820	25	145	40	106
11. 5000 lbs. 3–8–24 100 lbs. $CuSO_4$ 2000 lbs. NaCl	220	313	821	809	31	152	48	106
12. 5000 lbs. 3–8–24 3500 lbs. $BaCl_2$	240	459	809	828	25	176	56	114

* 50 gm. muck used.

† 25 gm. muck used.

may be explained in the work of Christensen.[4] He has shown that the speed of mannitol decomposition depends, in the main, on the reaction of the soil

[4] Christensen, H. R. 1923 Influence of soil condition on bacterial life and changes in soil substance: II. Ability of soil to break down mannite. *Soil Sci.* 15: 329–360.

and its buffer content, as well as on its content of easily soluble phosphoric-acid combinations. In other words, the differences in the power of soil to decompose mannitol depend primarily on differences in the chemical condition of the soil.

The application of lime caused a marked decrease in the liberation of CO_2 in the College muck, which had a pH of 7.0 (table 9). The addition of excess

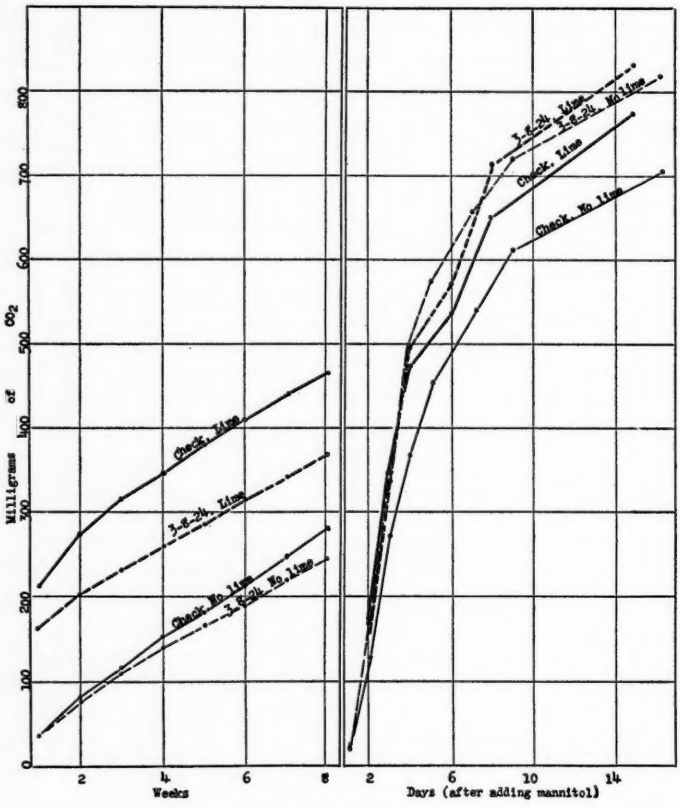


FIG. 1. CARBON DIOXIDE PRODUCTION IN CRANE MUCK

lime was toxic to the CO_2-producing organisms. In general, $CuSO_4$ applied at the rate of either 50 or 100 pounds per acre was neither harmful nor beneficial to the liberation of CO_2. Sodium chloride had a retarding effect on CO_2 production. This was true regardless of whether the salt was added with the 0–8–24 fertilizer, with the 3–8–24 fertilizer, or with $CuSO_4$.

Numbers of bacteria and fungi

The results obtained in the study of the effect of various mineral elements on the numbers of bacteria and fungi in College muck are shown in table 10. The 3–8–24 fertilizer alone or in combination with $CuSO_4$, NaCl, or $MnSO_4$

TABLE 10

Effect of various treatments on numbers of bacteria and fungi in College muck (pH 7.0)

TREATMENT PER ACRE	AVERAGE NUMBER OF COLONIES PER PLATE			
	Bacteria*	Fungi*	Bacteria†	Fungi†
1. Check	5.4	7.0	1.6	4.4
2. 1000 lbs. 0–8–24	3.8	2.2	2.0	2.6
3. 1000 lbs. 0–8–24 50 lbs. $CuSO_4$	4.6	1.6	0.8	11.4
4. 1000 lbs. 0–8–24 100 lbs. $CuSO_4$	30.4	28.6	0.8	9.2
5. 1000 lbs. 0–8–24 1000 lbs. NaCl	38.2	4.6	5.0	5.0
6. 1000 lbs. 0–8–24 2000 lbs. NaCl	144.2	17.0	4.0	3.8
7. 1000 lbs. 0–8–24 2000 lbs. NaCl 100 lbs. $CuSO_4$	131.6	97.6	8.2	3.0
8. 5000 lbs. 3–8–24	118.8	96.0	2.6	12.8
9. 5000 lbs. 3–8–24 100 lbs. $CuSO_4$	146.6	92.2	10.0	15.8
10. 5000 lbs. 3–8–24 2000 lbs. NaCl	160.4	130.2	30.8	8.0
11. 5000 lbs. 3–8–24 100 lbs. $CuSO_4$ 2000 lbs. NaCl	117.2	117.2	8.4	11.8
12. 5000 lbs. 3–8–24 400 lbs. $MnSO_4$	146.0	146.0	8.5	9.4

* Average of 5 plates inoculated with 1 cc. of dilution 1:4,000,000 and incubated 3 days. Counts made 10 weeks after experiment was set up.

† Average of 5 plates inoculated with 1 cc. of dilution 1:40,000,000 and incubated 2 days. Counts made 11 weeks after experiment was set up. Counts made on limed series used in CO_2 production studies.

was very effective in increasing the numbers of bacteria and fungi. In general, the 0–8–24 fertilizer, when applied alone, brought about a decrease in the numbers of bacteria and fungi, but with $CuSO_4$ and NaCl an increase was noted in most instances. Some of the materials used in this experiment may affect the plate counts directly through their effect on the degree of dispersion of the soil and of the microorganisms in the making of the suspensions for the dilutions. In other words, the effect of some of the chemicals on the counts may be more apparent than real. As a matter of interest in this connection a few data are presented in table 11 to show some of the differences that may be obtained from the addition of various chemicals when preparing a soil suspension preparatory to plating. Although the quantity of chemicals used in each instance in the latter experiment was greater than that in the experiments reported in table 10, the data illustrate the effect of soil treatment on dispersion of microorganisms, an effect which must be reckoned with in studies of this kind.

TABLE 11

Effect of dispersion on counts of bacteria and fungi in College muck (pH 7.6)*

TREATMENT†	BACTERIA	FUNGI
1. 200 cc. H_2O + 100 cc. 1 per cent NH_4OH	57.1	18.0
2. 200 cc. H_2O + 100 cc. *N* NaOH	3.8	9.0
3. 200 cc. H_2O + 100 cc. Sodium Silicate	43.3	3.0
4. 200 cc. H_2O + 100 cc. 1 per cent $CaCl_2$	4.5	1.3
5. 200 cc. H_2O + 100 cc. 1 per cent $Al_2(SO_4)_3$	2.3	27.0
6. 200 cc. H_2O + 100 cc. *N* HCl	2.5	2.1

* Figures represent the average of 6 plates inoculated with 1 cc. of dilution 1:40,000,000 and incubated 2 days.

† Solutions added to 50 gm. muck to make original suspension.

DISCUSSION

In regard to the fertilization of muck soils, it is not known whether the effect of certain metallic ions on plant growth is direct or whether it is exerted indirectly through chemical and microbiological changes produced in the soil. It was not expected that the investigations here reported would answer that question, but it was hoped that certain suggestions would be indicated. This problem necessarily deals with the relationships of microorganisms to soil fertility and involves a great number of interrelated factors, many of which are not well understood.

That microbiological changes in soil play an important part in determining crop yields, no one will doubt. The influences exerted by microorganisms are reflected in changes in the chemical, physical, and biochemical properties of soils. On the other hand, soil, as a culture medium, affects soil microorganisms both quantitatively and qualitatively. As higher plants develop in soils, many microbial activities are accelerated, and the place of most intense

activity becomes localized. Extensive development of microorganisms occurs in the rhizosphere during plant growth, and perhaps the most important effect is exerted by the organic substances arising from the roots themselves. It is in the rhizosphere that the effects of microorganisms on higher plants are particularly exerted. Microorganisms may favor the assimilation of nutrients by higher plants under certain conditions, and other microorganisms may be equally injurious under other conditions. It is evident that the addition of metallic ions to muck soils may affect this relationship. A just criticism, therefore, of the experiments reported here, is that they were carried on under laboratory conditions and in the absence of the growth of higher plants, but obviously most of these experiments could not be conducted under field conditions.

The effects of one organism upon the development of another may be of considerable importance in soil processes and must be considered in interpreting microbial response to muck treatment. Furthermore, some investigators are of the opinion that plant stimulants (of a vitamin nature) may arise through microbial action. The possibility exists, therefore, that when such materials as copper, sodium, boron, and manganese are added to muck soils they favor the development of these so-called plant stimulants, which are in turn responsible for increased yields and improved quality in crops.

SUMMARY AND CONCLUSIONS

The investigations here reported deal with the effect of various mineral elements on certain microbiological activities in muck soils. Materials such as $CuSO_4$, NaCl, $MnSO_4$, sulfur, boron, nitrogen, lime, phosphorus, and potash which are commonly applied to muck soils were used in these studies.

Microbial response was measured by studying ammonification, nitrification, carbon dioxide production, and changes in numbers of bacteria and fungi.

Nitrate accumulation (nitrates produced, less the amount assimilated and denitrified) in all but one of the acid mucks studied was favorably affected by the addition of $CaCO_3$. The beneficial effect is brought about by providing a more favorable environment for the nitrifiers by neutralizing the excess acidity and supplying an abundance of soluble calcium. Nitrification proceeded at a very slow rate below pH 5.5. Excess lime, particularly in the form of $Ca(OH)_2$, was in some cases detrimental to nitrate accumulation.

The 0–8–24 fertilizer gave small increases in nitrates on both limed and unlimed soils in most instances. The effects were not very pronounced, and it is believed that the benefits derived from that fertilizer when applied for crops are due to the direct effect of the fertilizer on the plant, rather than to any effect exerted indirectly by the microorganisms. The 3–8–24 fertilizer when used with lime gave consistent increases in the quantities of nitrates; but, without lime, increases were found in only about one-half the cases. Increases were expected where soluble nitrogen, in an easily nitrifiable form, was added, unless the nitrifiers were absent or were restricted in their activities

by some toxic condition. In some of the mucks, $(NH_4)_2SO_4$ nitrified too slowly to be of benefit to higher plants unless the nitrogen could be utilized in the ammonia form.

Results pertaining to the effects of $CuSO_4$ on ammonia and nitrate accumulation were not consistent. For the most part the results showed a retarding rather than a stimulating effect on both the limed and the unlimed mucks.

The addition of NaCl with lime resulted in decreased quantities of nitrates in all but one test. Without lime, increases were obtained in only about one-half of the cases. Where the nitrates were decreased, increases were noted in the quantities of ammonia accumulated. These increases apparently were due to a retarding effect of NaCl on the nitrifiers, thus permitting the ammonia to accumulate.

The addition of NaCl and $CuSO_4$ together with the 0–8–24 fertilizer caused a decrease in nitrate accumulation relative to the 0–8–24 fertilizer alone. When used with 3–8–24 fertilizer, NaCl and $CuSO_4$ brought about an increase (relative to 3–8–24 alone) in nitrates in the unlimed soils, but no consistent differences were found in the limed soils.

In general, the treatment consisting of NaCl, $CuSO_4$, and 0–8–24 fertilizer resulted in less nitrate accumulation than that in the check. Increases in nitrates occurred where the 3–8–24 fertilizer was added with NaCl and $CuSO_4$; the activities of the nitrifiers in these cases were not entirely prohibited by the NaCl and $CuSO_4$.

There is no conclusive evidence to indicate that $MnSO_4$ used at the rate of 400 pounds per acre had either a definite stimulating or a toxic effect on either the ammonifiers or the nitrifiers. Slight increases were observed in some instances and decreases in others.

Iron sulfate stimulated nitrification, but no appreciable effect (either beneficial or harmful) was noted from the use of KI, $BaCl_2$, Al_2SO_4, $ZnSO_4$, H_3BO_4, or Na_2SO_4. Heavier rates of application of some of these materials, undoubtedly, would have been toxic, but in these studies it was of more concern to note the effect of relatively light applications so they would be more comparable to ordinary field rates of application. The benefit obtained from $FeSO_4$ on nitrification was apparently due to the iron rather than the sulfate, inasmuch as several other compounds containing sulfate failed to stimulate either ammonification or nitrification.

Sulfur definitely stimulated nitrification in all the naturally alkaline mucks and also in those mucks that were heavily limed. The sulfur caused an increase in acidity, thereby reducing the toxicity of the alkali to the nitrifiers. No stimulating effect on nitrification was produced by the addition of sulfur to unlimed acid mucks, but an excess of sulfur decreased nitrate accumulation and increased ammonia accumulation. This indicates, as is commonly known, that the nitrifiers are much more sensitive to excess sulfur applications than are the ammonifiers.

The low nitrifying capacity or the slow rate of nitrification observed in some

of the mucks was apparently not due to a lack of a sufficient number of nitrifying organisms, because the addition of a fresh manure infusion (containing many active nitrifiers) failed to stimulate nitrification.

Studies concerning nitrification in solution cultures showed neither beneficial nor harmful effects from small applications of $MnSO_4$ and $CuSO_4$, but large quantities (0.01 gm. per flask, 50 cc. solution) of the latter were decidedly toxic.

Lime (in the form of $CaCO_3$) added to acid mucks increased the total quantities of CO_2 produced, but the differences were largely produced by the lime the first day after it was added; thereafter, the rate of CO_2 evolution was no greater than that from the unlimed soils. Evidence is presented to show that the increase in CO_2 was primarily due to a decomposition of the $CaCO_3$ added, rather than to the decomposition of the organic matter in the muck. Lime added to neutral and alkaline mucks caused a decrease in the evolution of CO_2.

Neither the 0–8–24 nor the 3–8–24 fertilizer was effective in increasing CO_2 production except in the Crane muck after mannitol was added; and then the 3–8–24 fertilizer gave decided increases.

No pronounced effect, either harmful or beneficial, on CO_2 production was observed with the addition of $CuSO_4$.

The production of CO_2 was retarded by the addition of NaCl. This was true whether the NaCl was added alone, with the 0–8–24 fertilizer, with the 3–8–24 fertilizer, or with $CuSO_4$.

The 0–8–24 fertilizer resulted in a decrease in the numbers of bacteria and fungi, according to the ordinary plate method of counting. Increases in numbers were obtained when either $CuSO_4$ or NaCl was used with the 0–8–24 fertilizer. The 3–8–24 fertilizer, alone or in combination with NaCl or $CuSO_4$, gave increases in numbers. Attention is directed to the dispersive effect of some of the treatments and to the effect that this might have directly on the plate counts.

EFFECT OF SALINE WATER ON MEDITERRANEAN LOESS SOILS

M. PUFFELES[1]

Government Central Laboratories, Jerusalem

Received for publication November 15, 1938

Research workers agree that the Beersheba soils are of the loess type, which is suitable for agricultural purposes.

Range (11) was the first investigator to deal scientifically with the loess deposits in the Beersheba basin (southern Palestine). After careful study he came to the conclusion in 1922 that these loess soils are sediments of genuine aeolian origin which have been redeposited in part by running water. In 1932, he reattacked the problem, collecting additional data by analyzing a few soils. The result of this investigation confirmed his previous findings. His experimental work has led him to believe that the soil possesses good physical properties and is rich in lime, but is deficient in organic matter and such plant nutrients as K and P_2O_5.

Menchikowsky (9) divides the Beersheba soils into two classes: the loess soils and the desert gravel soils. The dividing line between these two types of soils is represented by a perpendicular dropped from a point midway between Gaza and the Dead Sea (slightly to the east of Beersheba) to the Rafa-Akaba boundary line. The loess soils in this district bear some resemblance to the European loess soils as to color, structure, texture, and stratification. These soils are particularly rich in lime, naturally well drained, and readily permeable to air and water.

Strahorn (12) thinks that the Beersheba soils can be considered to be of the loess type, formed by the settling of fine dust particles which have been carried by winds from a southern direction. The entire profile of the type seems to be calcareous. Furthermore, he adds that the drainage conditions in these soils are good.

IRRIGATION POSSIBILITIES

The development of agriculture in the Beersheba area is conditional on the discovery of subterranean water sources of a suitable nature for the irrigation of plants. Experimental borings made recently by the Government Department of Development have shown that there is water under pressure in this district. The disadvantage of the water thus found is its high saline content. But as agricultural science has shown, saline water can be used for the irrigation of halophytic plants such as date palm, sugar cane, beets, grapes, and cotton.

[1] The author wishes to express his thanks to the government analyst, G. W. Baker, for his advice and corrections, and to S. Adler for her assistance in the analytical work.

The aim of this investigation was to determine whether the use of these saline waters for irrigation would cause deterioration of the soil.

EXPERIMENTAL

A complete analysis of the saline water obtained from the government borings, analyses of four soils before and after irrigation with saline water, and tests on the alkalinity of the drainage water were made.

The soils examined have the following characteristics:

The surface layers of the four profiles are light-brown loess.

The soils are nearly free from gravel and have a slightly compact structure.

The subsoils are also light-textured loam. The fragments of the subsoil do not show any cementation, and they are easily broken by hand.

The entire profiles of these types are calcareous in origin.

As the soils were not homogeneous, the samples were collected by layers. The following methods were used for the various analyses: The mechanical

TABLE 1

Analysis of water

DETERMINATIONS		PROBABLE COMBINATIONS	
	p.p.m.		*p.p.m.*
Chlorine (Cl)	3,630	$CaCO_3$	190
Calcium (Ca)	434	$CaSO_4$	1,217
Magnesium (Mg)	198	$MgSO_4$	417
Sulfates (SO_4)	1,192	$MgCl_2$	447
Carbonates (CO_3)	114	NaCl	5,440
Nitrates (NO_3)	traces	$NaNO_3$	traces
Total solids at 140°C.	7,690	Total	7,711

analyses was made according to the Sudan method worked out by Beam (2). The pore space is expressed by the formula $\frac{a-b}{a} \times 100$, where a = true specific gravity and b = the apparent specific gravity. The water-holding capacity was determined according to Keen and Raczkowski (7), the permeability was expressed as hours per liter. The water extract in the chemical analyses was made according to Gedroiz (4); the P_2O_5, K_2O, $CaCO_3$, and total nitrogen were determined according to the A. O. A. C. method (1); and the exchangeable bases, according to Hissink (5).

The results of the analysis of water from the borings are given in table 1. It will be observed that the salt content is high, the NaCl alone being 5,440 p.p.m., and the total solids at 140°C. 7,690 p.p.m.

As shown in table 2, the moisture and clay contents of the soils increase with depth, except in profile IV, the middle layer of which has the most clay, and the bottom layer, the least moisture.

The results of the physical analyses of the soil samples, shown in table 3, indicate that the soil is of a type which is highly permeable to water and air.

Analyses of the water extracts (table 4) show that the soils have a slight alkaline tendency and are not rich in harmful salts, with the exception of profile

TABLE 2

Mechanical composition and moisture content of soil samples

	DEPTH	MOISTURE AT 100°C.	CLAY	SILT	FINE SAND	COARSE SAND
	cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	surface	2.8	19.4	10.2	67.0	3.4
	10–20	3.1	25.4	15.0	57.0	2.6
	20–60	3.3	29.4	11.6	55.0	4.0
II	0–30	2.4	18.4	12.0	53.0	16.6
	30–60	2.5	19.0	7.8	27.8	45.4
	60–100	2.5	19.4	10.6	27.0	43.0
	100–150	2.7	22.4	9.0	32.6	36.0
III	0–30	3.7	27.6	15.8	45.6	11.0
	30–150	4.0	31.4	17.6	30.0	21.0
IV	0–30	3.2	22.4	11.8	58.6	7.2
	30–60	3.2	31.8	15.8	39.6	12.8
	60–100	2.8	25.0	16.4	30.6	28.0

TABLE 3

Physical analyses of soil samples

	DEPTH	TRUE SPECIFIC GRAVITY	APPARENT SPECIFIC GRAVITY	PORE SPACE	WATER-HOLDING CAPACITY	PERMEABILITY*
	cm			*per cent*	*per cent*	
I	surface	2.321	1.324	42.9	36.5	38.6
	10–20	2.289	1.324	42.1	43.6	200
	20–60	2.269	1.364	39.9	44.2	750
II	0–30	2.299	1.453	37.2	33.4	200
	30–60	2.533	1.506	44.8	31.7	180
	60–100	2.533	1.438	43.2	36.2	170
	100–150	2.520	1.426	43.4	39.5	220
III	0–30	2.458	1.384	44.5	36.1	500
	30–150	2.545	1.422	44.1	37.0	700
IV	0–30	2.470	1.440	41.5	38.7	250
	30–60	2.470	1.443	41.7	42.9	700
	60–100	2.533	1.468	42.0	37.8	270

* Hours per liter.

III, 0–30 cm., in which the chlorine content is high (0.34 per cent). The rest of the analytical data in table 4 verify the fact that the soils are poor in such nutrients as K_2O, P_2O_5, and total nitrogen. The lime content is high, an

advantage in that lime tends to preserve the stability of the natural qualities of loess soils and prevents the deterioration processes which result in the

TABLE 4

Analyses of water and hydrochloric acid extracts, calcium carbonate and total nitrogen of soil samples

DEPTH		WATER EXTRACT 1:5		HCl EXTRACT		$CaCO_3$	TOTAL NITROGEN KJELDAHL
		Cl	pH	P_2O_5	K_2O		
cm.		*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
I	surface	0.010	7.8	0.077	0.22	14.5	0.043
	10–20	0.034	7.95	0.087	0.22	20.7	0.039
	20–60	0.068	8.1	0.112	0.28	24.0	0.020
II	0–30	0.012	7.9	0.063	0.14	28.3	0.036
	30–60	0.006	8.1	0.071	0.06	33.3	0.031
	60–100	0.009	7.9	0.066	0.07	38.7	0.031
	100–150	0.008	8.1	0.041	0.12	31.9	0.028
III	0–30	0.34	7.4	0.056	0.18	26.7	0.056
	30–150	0.012	7.9	0.046	0.16	30.2	0.031
IV	0–30	0.006	7.9	0.056	0.24	21.0	0.034
	30–60	0.018	7.9	0.054	0.12	31.9	0.034
	60–100	0.011	8.0	0.046	0.14	38.3	0.028

TABLE 5

Exchangeable bases of soils

DEPTH		Ca	Mg	K	Na	TOTAL BASE EXCHANGE
cm.		*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
I	surface	11.52	2.14	0.77	0.20	14.63
	10–20	11.52	2.58	0.77	0.30	15.17
	20–60	9.80	3.03	0.77	0.30	13.90
II	0–30	13.22	2.12	0.68	0.20	16.22
	30–60	11.00	2.40	0.70	0.20	14.30
	60–100	11.52	3.00	0.77	0.25	15.54
	100–150	13.50	2.14	0.77	0.28	16.69
III	0–30	14.00	2.58	0.50	0.18	17.26
	30–150	14.62	2.70	0.70	0.30	18.32
IV	0–30	13.50	2.16	0.66	0.26	16.58
	30–60	14.60	2.20	0.77	0.30	17.87
	60–100	11.52	2.08	0.80	0.30	14.70

formation of pan (Nazaz). Such formation has been dealt with by the author in a previous article (10).

The base-exchange contents of the soil samples are shown in table 5. The total base exchange in all profiles varies from 14–18 m.e. and consists mostly of the calcium ion.

The effect of the saline water on the soils was determined by the following method. The soils were packed into cylindrical containers, according to their original profiles, every centimeter of depth being represented by one milli-

TABLE 6

Analyses of pH value or sodium carbonate content in the drainage water

PROFILE	pH				Na_2CO_3*							
	March 10	March 24	June 7	June 21	May 5	May 19	June 2	June 16	June 30	July 13	July 27	Aug. 11
I	7.85	8.2	8.6	>8.8	0.40	0.41	0.39	0.40	0.42	0.36	0.42	0.41
II	7.8	8.15	8.5	>8.8	0.35	0.37	0.36	0.37	0.35	0.38	0.37	0.39
III	7.9	8.0	8.4	>8.8	0.35	0.35	0.36	0.33	0.35	0.37	0.37	0.37
IV	7.75	8.1	8.6	>8.8	0.32	0.35	0.33	0.35	0.34	0.35	0.36	0.38

* Grams per liter.

TABLE 7

Permeability and exchangeable bases of soils

AVERAGE OF PROFILES		PERMEABILITY*	EXCHANGEABLE BASES				
			Ca	Mg	K	Na	Total
			m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*
I	Before treatment with saline water	329	10.95	2.58	0.77	0.27	14.56
	After 6-month treatment with saline water	2,823	6.00	1.28	0.15	6.89	14.32
II	Before treatment with saline water	192	12.32	2.42	0.73	0.22	15.70
	After 6-month treatment with saline water	2,742	3.40	0.81	0.23	11.51	15.95
III	Before treatment with saline water	600	14.31	2.64	0.60	0.24	17.79
	After 6-month treatment with saline water	2,909	4.00	1.30	0.25	12.45	18.00
IV	Before treatment with saline water	407	13.20	2.15	0.74	0.29	16.39
	After 6-month treatment with saline water	2,823	5.60	1.40	0.24	8.78	16.02

* Hours per liter.

meter in the cylinder. A perforated false bottom over which is fitted a filter paper allows the drainage water to pass to a funnel-shaped outlet without loss of soil through the perforations. The saline irrigation water was added in measured quantity once every 2 weeks, and the water which drained out was examined for its alkalinity content. The average results from duplicate cylinders for each profile are given in table 6.

It will be observed from table 6 that the minimum rise in pH value in the first 3 months was from 7.9 to more than 8.8. The sodium carbonate, which accumulated in time, was determined once every 2 weeks for a period of 6 months. In view of the fact that there was not much difference in the Na_2CO_3 content from one period to another, the soils were taken from the cylindrical vessel at the end of the 6 months and dried until they were fit for crushing. Then they were thoroughly mixed, dialyzed until chlorine free, and finally examined for permeability and exchangeable bases. The results, in comparison with those for the unirrigated soils, are given in table 7. It is quite evident that the permeability decreased markedly in all cases and that most of the exchangeable calcium has been replaced by sodium in the irrigated soils.

DISCUSSION

Although agricultural science has shown that saline water can be used, within limits, for the successful cultivation of certain salt-resistant plants, as described by Kearney and Scofield (6) and by Foaden (3), two factors must be considered; namely, the direct effect of saline water on the plant; and the effect of saline water on the soil, which in turn will affect the growth of plants. It is a fact that plants can withstand much higher salt concentration than that causing soil deterioration, but any effect on the soil is bound, in time, to be reflected in the growth of plants. Hence, there are two possible results to plants of the use of very saline water for irrigation: either the salts will accumulate and finally prevent plant growth; or, as in our case, the salts will not accumulate under favorable climatic and drainage conditions, but, instead, an alkaline soil will be formed which is unsuitable for the growth of plants. In this investigation we have an extreme case with a very saline water. How far the salinity would have to be reduced by mixing the saline water with soft water, by adding gypsum to the water or applying gypsum to the soil, or by passing through the water an electric current, which causes the separation of the adsorbed and combined salts—a recent method tried out successfully in the Punjab Irrigation Research Institute of Lahore—is a matter for further experiments. Kelley (8), as well as Taylor[2] in his experiments at the Punjab Research Institute, gives a maximum of 600 p.p.m. sodium salts for an irrigation water irrespective of the presence of calcium in the soil and water. On the evidence available, however, it is not considered that this limit could be generally applied also in Palestine.

SUMMARY

Investigations made on the loess soils of the Beersheba area show that this type of soil is highly permeable to air and water and possesses good physical properties. The soil is poor in nutrients. The presence of large quantities of lime tends to preserve the stability of the natural properties of the soil and to decrease the rate of deterioration. If the very saline water that is available

[2] Personal communication.

is used for irrigation, under favorable climatic and drainage conditions, such as obtain in this area, the salts will not accumulate, but, instead, an alkaline soil will, in time, be formed by base exchange. This soil will eventually be useless for agricultural purposes.

REFERENCES

(1) Association Official Agricultural Chemists 1935 Official and Tentative Methods of Analysis, ed. 4. Washington, D. C.

(2) Beam, W. 1911 The mechanical analysis of arid soils. *Cairo Sci. Jour.* 5: 107–119.

(3) Foaden, G. P. 1897 Cotton culture in Egypt. U. S. Dept. Agr. Off. Exp. Stas. Bul. 42.

(4) Gedroiz, K. K. 1926 Chemische Bodenanalyse. Gebrüder Borntraeger, Berlin.

(5) Hissink, D. J. 1923 Method for estimating adsorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.

(6) Kearney, T. H., and Scofield, C. S. 1936 The choice of crops for saline land. U. S. Dept. Agr. Cir. 404.

(7) Keen, B. A., and Raczkowski, H. 1921 The relation between the clay content and certain physical properties of a soil. *Jour. Agr. Sci.* 11: 441–449.

(8) Kelley, W. P. 1934 The formation, evolution, reclamation and the adsorbed bases of alkali soils. *Jour. Agr. Sci.* 24: 77.

(9) Menchikowsky, F. 1937 Reclamation of the Negeb (Beersheba-area). *Palestine and Middle East* 9: 475–478.

(10) Puffeles, M. 1937 Some data on the Mediterranean red soils. *Soil Sci.* 44: 167–174.

(11) Range, P. 1922 Die Küstenebene Palästinas. *Gesell. Palästina-Forsch. 8 Veröffentl.* 9–11.

(12) Strahorn, A. T. 1928 Soil Reconnaissance of Palestine. Reports of the Experts Submitted to the Joint Palestine Survey Commission, pp. 195–196. Daniels Ptg. Co., Boston.

PLANT GROWTH AND THE BREAKDOWN OF INORGANIC SOIL COLLOIDS[1]

W. A. ALBRECHT, ELLIS R. GRAHAM, AND CARL E. FERGUSON[2]

Missouri Agricultural Experiment Station

Received for publication November 28, 1938

The recent use of electrodialyzed colloidal clay as a plant medium[3] lends itself to quantitative chemical scrutiny of the clay and plant behaviors, particularly their interactions. Such preliminary scrutiny in carefully controlled experiments suggests that plant growth may be influential in bringing into molecular, or ionic, form some part of the colloidal clay even though it was subjected at the outset to prolonged electrodialysis to remove, or reduce to a minimum, these forms. The following study is an examination of this hypothesis by analysis of soybean plants for silicon, aluminum, and iron after a growth period of 5 weeks in comparison with similar analyses of the clay and the seed at the outset. Such a chemical balance sheet points rather forcibly to the truth of such a belief.

PLAN OF STUDY

Clays of 200 mμ and smaller diameters were electrodialyzed until they released no more cations, and then were supplied with different quantities of calcium, magnesium, and barium by titration with the hydroxides of these elements so as to produce a nearly neutral clay. Clays were so prepared and such amounts taken for mixture with a leached coarse quartz sand as to provide constant levels of calcium, while the magnesium was varied and the barium fluctuated as its reciprocal. Two different levels were used, the second double the first. These were 10 and 20 m.e. for the calcium; 10, 5, and 0, and 20, 10, and 0 m.e. for the magnesium; and 0, 5, and 10, and 0, 10, and 20 m.e. for the barium. Thus a total of 20 m.e. per 50 plants was provided in the lower level, and 40 m.e., in the higher level.

No other cations were supplied to the clay; and after the addition of these cations, the ultrafiltrate was shown by tests to be free of all ions, with the exception of a possible trace of silica. Soybeans were sprouted and planted

[1] Contribution from the department of soils, Missouri Agricultural Experiment Station Journal Series No. 583.

[2] Professor, instructor, and graduate scholar, department of soils, College of Agriculture, University of Missouri, Columbia, Missouri.

[3] Albrecht, W. A., and McCalla, T. M. 1938 The colloidal clay fraction of soil as a cultural medium. *Amer. Jour. Bot.* 25: 403–407.

in the clay-sand mixtures and grown for 5 weeks, after which the complete crop was removed, dried, weighed, and prepared for analysis. One of the clay suspensions without sand was submitted to carbon dioxide bubbling for an hour daily for 6 weeks. The H-clay from the same source and of similar electrodialytic treatment had been previously analyzed.[4]

Thus with an inventory of the silicon, aluminum, and iron content of the clay at the outset coupled with the supply of these in the seed, and then with the analysis of the crop and the CO_2-treated clay at the close, it was possible to measure the effect of the crop in removing these elements from the clay as contrasted to their liberation from the clay under carbonic acid solution.

EXPERIMENTAL RESULTS

The balance sheets for the various elements are assembled in tables 1 and 2, table 1 giving the amounts at the outset, and table 2 the amounts taken up by the crop and the percentage of the initial supply represented by the crop contents.

Since the silicon, aluminum, and iron were supplied by the clay in non-electrodialyzable form, and in very small and relatively insignificant amounts by the seed, the mobilization of these elements from the clay to the crop will serve well as indexes of the influence of the crop growth on the breakdown of the colloidal clay. Though these elements were originally removed by electrodialysis in the production of the hydrogen clay and were not present in the ultrafiltrate after this was titrated to the neutral condition carrying calcium, magnesium, and barium; yet, as an average of all six trials, the crop took up 2.21 per cent of the silicon, 1.95 per cent of the aluminum, and 3.14 per cent of the iron present in the original clay by total analysis. Doubling the amount of clay increased the crop's total content of silicon 1.79 times, of aluminum 3.00 times, and of iron 1.91 times.

In contrast to this generous liberation of silicon, iron, and aluminum from the clay by the plants is the insignificant conversion of these elements into the soluble form by the treatment of the clay with carbonic acid bubbling through it for 6 weeks, as shown in table 2. These data point out that the effects of the plant on the clay are more drastic than is the simple condition set up by the carbon dioxide saturation of the clay suspension the final pH of which was 6.0–6.3, in contrast to an initial pH of 6.9.

If plant growth is effective in bringing about disintegration of the colloidal clay with the release of iron, aluminum, and silicon in the ionic form, the other positive ions in the clay lattice might well be expected to be similarly released. Since the crop which took up these quantities of aluminum, iron, and silicon was grown without additions of exchangeable potassium, the crop analysis for this item should contribute some information.

If we assume that during this experiment physiological conditions were

[4] Marshall, C. E. 1935 Layer lattice and the base-exchange clays. *Ztschr. Krist.* A91: 433–449.

normal for maximum potassium consumption by the plants, then we might conclude from the data for the potassium balance in tables 1 and 2, that the clay delivered no potassium to the plant. The potassium content of the crop agreed closely, on the average, with that supplied by the seed. Delivery by

TABLE 1

Quantities of various elements initially present in clay and additional quantities supplied by titration and seed

PLOT NUMBER	TITRATED CLAY CONTENT			TOTAL CLAY	INITIAL CLAY CONTENT						SUPPLIED BY				
											Titration		Seed		
	Ca	Mg	Ba		Si	Al	Fe	K	Mg	Ca	Ca	Mg	Ca	Mg	K
	m.e.	*m.e.*	*m.e.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1 & 2	10	0	10	29.4	6,762	3,754.3	1,684.6	296	347	0.052	200	0	12.2	16.7	171
3 & 4	10	5	5	29.4	6,762	3,754.3	1,684.6	296	347	0.052	200	60	12.2	16.7	171
5 & 6	10	10	0	29.4	6,762	3,754.3	1,684.6	296	347	0.052	200	120	12.2	16.7	171
7 & 8	20	0	20	58.8	13,524	7,508.6	3,369.2	592	694	0.104	400	0	12.2	16.7	171
9 & 10	20	10	10	58.8	13,524	7,508.6	3,369.2	592	694	0.104	400	120	12.2	16.7	171
11 & 12	20	20	0	58.8	13,524	7,508.6	3,369.2	592	694	0.104	400	240	12.2	16.7	171
CO_2-treated clay.....				1.96	450.8	250.2	112.3								

TABLE 2

Quantities of the various elements taken by the crop and liberated from the clay by carbon dioxide treatment

PLOT NUMBER	Si	Al	Fe	K	Mg	Ca	PER CENT OF CLAY SUPPLY					
							Si		Al		Fe	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*						
In crop												
1 & 2	196.2	60.8	58.8	172	20.2	66.2	2.90		1.62		3.49	
3 & 4	103.6	51.0	52.7	185 } 173	48.3	79.5	1.53 }	2.33	1.36 }	1.56	3.12 }	3.21
5 & 6	173.8	64.2	50.8	162	100.1	89.8	2.57		1.71		3.02	
7 & 8	338.6	230.9	140.9	147	19.5	76.8	2.50		3.07		4.18	
9 & 10	200.5	131.4	75.8	203 } 178	92.2	87.6	1.48 }	2.09	1.75 }	2.34	2.25 }	3.07
11 & 12	310.7	164.7	93.9	185	178.1	114.1	2.29		2.19		2.79	
In filtrate												
CO_2-treated clay.....	0.9	0.4	0.2				0.2		0.16		0.18	

the clay of quantities of potassium amounting to 2 or 3 per cent of the 296 and 592 mgm. contained in the clay might be readily masked, however, by the 171 mgm. supplied by the seed. It is possible, on the other hand, that the location of the potassium within the clay particle is such that iron, aluminum, and silicon may be released without necessarily dislodging the potassium extensively.

Though the calcium and magnesium in the clay were also studied quantitatively, the question of whether they came out of the crystal lattice of the colloidal clay could not be answered with certainty. If magnesium were released at the rate indicated for iron, aluminum, and silicon, namely, 2 or 3 per cent, the minimum figure would supply only 7 and 14 mgm. of magnesium for the lighter and heavier clay dosages, respectively, to a crop that was started with 16.7 mgm. in the seed and consumed, in addition, 60 to 70 per cent of the 60, 120, or 240 mgm. of the exchangeable magnesium added to the clay. The large demand on magnesium for good crop growth is in excess of what the clay could offer even if it broke down at a rate far beyond that suggested by the release of iron, aluminum, and silicon.

It is interesting to note, however, that the crop grown on electrodialyzed colloidal clay given "no treatment" of magnesium had a magnesium content in excess of that in the planted seed by 3 to 3.5 mgm. This excess might be considered as possible release from the lattice. If these are accurate figures, they would indicate a breakdown equivalent of 1 or 0.5 per cent—not so far removed from the 2 or 3 per cent suggested by the liberation of iron, aluminum, and silicon.

The calcium determinations of the study fail to give any suggestion regarding possible clay breakdown. Since the total calcium consumed by the plants was far in excess of the total in the lattice of the clay, these large quantities cover thoroughly any indication of movement from the crystal to the adsorbed form in which it might be taken by the plants.

SUMMARY

Complete analyses of the soybean seed and colloidal clay at the outset and of the crop at the close of the experiment, provide a balance sheet of the movements by both the exchangeable and the nonexchangeable cations in the colloidal clay. This study suggests that a clay electrodialyzed free of its cations, then saturated with only barium, magnesium, and calcium to a pH of 6.9 or 7.0 and planted to soybeans, is broken down by the plant growth with release of the silicon, aluminum, and iron to the extent of 2 or 3 per cent of the total in the clay. This is the release from a clay of extreme mineralogical stability that was first submitted to the extracting force of electrodialysis for periods as long as 3 weeks, and then to plant root contact for less than 6 weeks. Increasing amounts of clay meant correspondingly increased amounts of these three cations taken by the plants.

The percentages of silicon, aluminum, and iron released by the clay in these studies are so low that if the total calcium, potassium, and magnesium in the clay were set free at the same rate, their amounts would be insignificant in providing the needs of the plants growing on them. These data suggesting colloidal clay breakdown by the plant offer no encouragment to the belief that the plant can solve its fertility needs in bases by attacking the nonexchangeable stores in the clay fraction of the soil, even though it might obtain enough iron to supply its needs for this single nutrient.

STUDIES ON THE NITROGEN, PHOSPHORUS, AND MINERAL REQUIREMENTS OF ALFALFA[1]

A. W. BLAIR AND A. L. PRINCE[2]

New Jersey Agricultural Experiment Station

Received for publication December 2, 1938

It is well known that alfalfa draws heavily upon certain of the mineral elements in the soil, especially calcium and potassium. Less is known of its phosphorus requirements.

Following some experiments with potatoes in which phosphorus was the limiting factor, the work was continued along somewhat similar lines with alfalfa.

The soil is a Sassafras loam of fair quality, though not quite typical Sassafras because of its nearness to the Penn (Triassic red shale formation). Starting in 1924, potatoes were grown continuously for 10 years on $\frac{1}{4}\frac{1}{4}$-acre plots supplied with a fertilizer containing different ratios of phosphorus, the analysis varying from 4–0–4 to 4–16–4 in steps of 4 per cent P_2O_5. In 1931 the nitrogen was raised to 5 per cent; and in 1932 the potash, to 7 per cent. The fertilizer was used at the rate of 1,600 pounds to the acre, and lime was used to give a pH near 6.0, since potato scab was not a disturbing factor in this case.

The results with potatoes were largely negative, very little increase in yield being obtained as the phosphorus was increased. The results from this part of the work need not be discussed here, other than to say that potatoes (not including vines) remove very little phosphorus from the soil, only about 18 pounds P_2O_5 for a 200-bushel crop. As the average yield of potatoes on these plots was less than 150 bushels to the acre, the total amount of P_2O_5 removed for the 10-year period probably did not much exceed 130 pounds to the acre.

Following the potatoes in 1933, wheat was seeded without further fertilizer treatment. The wheat crop of 1934 was unusually large, but again the difference between the phosphorus-treated plots and those that received no phosphorus was insignificant. Following the wheat, the land was prepared for alfalfa and seeded in August 1934. The amount of fertilizer was reduced to the equivalent of 800 pounds an acre annually, and the use of commercial nitrogen was discontinued with the exception of a light application at the time

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[2] The authors are indebted to A. L. Grant, who worked under W.P.A. Project 1077–12, Research and Special Survey and Plant Food Resources, for a large part of the CaO and MgO determinations.

of seeding the alfalfa. Lime has been applied at intervals to keep the soil at a pH of about 6.0 to 6.5.

A careful study of table 1 shows some differences in favor of the phosphorus treatment. On the other hand, in three cases, the average for the plots that received the maximum phosphorus treatment is slightly below the average for the four check plots.[3] The greatest difference in favor of the maximum phosphorus treatment over the check treatment was 290 pounds of hay for the third cutting in 1937, but in this case the 0–4–7 treatment gave a lower average than the check, and the 0–8–7 gave only 48 pounds more than the check.

TABLE 1

Yield of dry alfalfa hay, in pounds to the acre, from plots with varying phosphorus treatments, 1935–1938

FERTILIZER TREATMENT*	1935		1936		1937			1938			ANNUAL AVERAGE
	Cuttings										
	First	Second	First	Second	First	Second	Third	First	Second	Third	
0–0–7	3,346	2,723	4,574	2,634	4,162	2,951	1,701	4,784	3,118	2,379	8,093
0–4–7	3,358	2,707	4,581	2,637	3,848	2,823	1,589	4,816	3,110	2,127	7,899
0–8–7	3,367	2,742	4,374	2,522	3,978	2,742	1,749	5,057	3,032	2,239	7,951
0–12–7	3,363	2,871	4,657	2,621	3,922	2,790	1,835	5,056	3,153	2,396	8,166
0–16–7	3,508	2,703	4,542	2,698	4,242	3,047	1,991	4,795	3,231	2,160	8,229
Av. for the four phosphorus treatments	3,399	2,756	4,539	2,620	3,998	2,851	1,791	4,931	3,132	2,231	8,061

* On the basis of 800 pounds to the acre annually.

Comparison of the averages for the four check plots with the averages for the four different phosphorus treatments, shows that the differences are, for the most part, negligible. The 4-year average for all the phosphorus treatments is slightly less than the 4-year average for the four check plots. On the basis of the 4-year averages, some increase in yield is noted for the highest two applications of phosphorus, but when the cost of this extra phosphorus is taken into account, it is found that the increased expenditure for fertilizer is not justified.

PERCENTAGE OF P_2O_5 IN ALFALFA HAY

Table 2 shows the percentage of P_2O_5 in the hay for the various treatments throughout the 4 years. The variations are not great, but comparison of the averages for the four treated plots with the averages for the four check plots shows a slight tendency toward an increase in percentage of P_2O_5 with increase in amount of phosphorus applied. In some cases the increase is

[3] The figures for the check plots represent the average of four plots distributed over the area under consideration. The figures for each of the other treatments represent the average for two plots located some distance apart.

pronounced. For example, in the first cutting in 1937 for the 0–16–7 treatment, the increase was 37 per cent, and in the first cutting of 1938, the increase for the same treatment was 49 per cent. In an earlier paper in connection with other crops (3), attention was directed to this tendency. There is a slight tendency for the percentage to be lower in the first cutting than in the second and third cuttings, though this is not true for the crop of 1937. If 0.67 per cent is taken as a fair average for the P_2O_5 in alfalfa hay from land that has received phosphorus treatment, a ton of hay removes a little over 13 pounds P_2O_5.

TABLE 2

Percentage of P_2O_5 in dry alfalfa hay grown with varying phosphorus treatments, 1935–1938

FERTILIZER TREATMENT	1935		1936		1937			1938			AVERAGE FOR 10 CUTTINGS
	Cuttings										
	First	Second	First	Second	First	Second	Third	First	Second	Third	
0–0–7	0.422	0.702	0.570	0.659	0.605	0.615	0.633	0.525	0.740	0.656	0.613
0–4–7	0.383	0.739	0.565	0.652	0.622	0.637	0.689	0.524	0.699	0.647	0.616
0–8–7	0.406	0.705	0.655	0.694	0.694	0.652	0.704	0.586	0.762	0.665	0.652
0–12–7	0.432	0.711	0.683	0.682	0.795	0.671	0.661	0.683	0.789	0.780	0.689
0–16–7	0.450	0.702	0.783	0.708	0.830	0.673	0.682	0.783	0.845	0.763	0.722
Av. for the four phosphorous treatments	0.418	0.714	0.672	0.684	0.735	0.658	0.684	0.644	0.774	0.714	0.670

POTASH CONTENT OF ALFALFA HAY

The potash content of alfalfa hay was discussed in a previous paper (4). The percentage varies somewhat with the time of cutting and the purity of the hay. Van Slyke (10, p. 714) gives 2.1 per cent K_2O for alfalfa hay. Unpublished analyses from this station show an average of about 2 per cent K_2O, ranging from 1.4 to 2.7 per cent. If we take the analysis given by Van Slyke, the amount of K_2O removed by a ton of hay is 42 pounds. To meet this loss, 350 pounds of an alfalfa fertilizer carrying 12 per cent K_2O would be required, none being left to replace that lost through leaching and erosion. The amount suggested is much more than the average application for alfalfa. It seems very evident, therefore, that Eastern alfalfa growers have too lightly considered the potash requirements of alfalfa.

The soils on which alfalfa is usually grown in New Jersey, contain 10 to 15 times as much K_2O as P_2O_5, and the question may well be raised: Why apply so much potassium when the soil is so abundantly supplied with this constituent? The answer is undoubtedly to be found in the high insolubility of potash in the soil minerals. Certainly these materials are much more insoluble in the soil solution than are phosphorus-bearing materials.

PERCENTAGE OF ASH, MgO, AND CaO IN ALFALFA HAY

Table 3 shows the percentage of ash, CaO, and MgO in the hay of the first cutting for the 4-year period. In these figures there is little to indicate that these constituents have been significantly influenced by the phosphorus treatment.

The percentage of ash is fairly constant, about 7 per cent. A few exceptions may possibly be attributed to particles of soil adhering to the sample. The percentage of CaO in the hay is higher for 1935 than for the following years, and the percentage of MgO is slightly higher for 1937 and 1938 than for 1935 and 1936. The authors have no explanation for this. The high percentage of CaO as compared with MgO, throughout, is of special interest. According to the figures for 1935, a ton of alfalfa hay would remove from the soil more

TABLE 3

Percentage of ash, CaO, and MgO in dry alfalfa hay grown with varying phosphorus treatments, 1935–1938

(First cutting of each year)

FERTILIZER TREATMENT	1935			1936			1937			1938		
	Ash	CaO	MgO	Ash	CaO	MgO	Ash	CaO	MgO	Ash	CaO	MgO
0–0–7	7.15	3.29	0.383	6.83	1.98	0.350	7.40	2.27	0.457	7.57	2.06	0.616
0–4–7	7.20	3.25	0.398	7.15	2.09	0.398	7.00	1.83	0.415	7.36	1.98	0.565
0–8–7	7.11	3.07	0.360	7.25	2.23	0.356	7.32	2.17	0.421	7.02	2.12	0.500
0–12–7	7.39	3.01	0.370	8.14	2.28	0.367	7.80	2.38	0.410	6.79	1.89	0.703
0–16–7	6.89	3.08	0.359	6.87	2.01	0.326	7.57	1.96	0.393	6.30	1.87	0.524
Av. for the four phosphorus treatments	7.15	3.10	0.372	7.35	2.15	0.362	7.42	2.09	0.410	6.86	1.96	0.573

than 60 pounds of CaO and less than 8 pounds of MgO. In comparison, a ton of wheat (grain) would remove 1 pound or less of CaO; and the straw from this grain, about 7 pounds of CaO. The importance of having feeding materials that are high in calcium is well known. Ames and Boltz (1) have reported on the nitrogen and mineral constitutents of the alfalfa plant; and Brown (5), for Connecticut, and Archibald, Nelson, and Bennett (2), for the U. S. Department of Agriculture, have discussed the effects of fertilizers on the chemical composition of pasture grasses. Forbes, Whittier, and Collison (6) report the mineral nutrients in bluegrass.

NITROGEN IN ALFALFA HAY

Table 4 shows the annual removal of nitrogen by the hay crop for the 4-year period. The phosphorus treatment had virtually no effect on the amount of nitrogen removed through the hay. With the exception of the crop for 1935, the first cutting of hay removed distinctly more nitrogen than did the later cuttings. This is due to the reduced yields for the second and third cuttings.

The annual acre yield of nitrogen for the 4-year period is about 220 pounds, two-thirds to three-fourths of which was probably obtained from the atmosphere (7).

Table 5 shows the average percentage of nitrogen in the hay from the four check plots in comparison with the average for the hay from the four that receive phosphorus. Comparison of the two rows of figures shows that the percentage of nitrogen has not been significantly influenced by the phosphorus

TABLE 4

Nitrogen, in pounds to the acre, removed through alfalfa hay grown with varying phosphorus treatments, 1935–1938

FERTILIZER TREATMENT	1935		1936		1937			1938			ANNUAL AVERAGE
	Cuttings										
	First	Second	First	Second	First	Second	Third	First	Second	Third	
0–0–7	82.5	80.1	120.3	75.4	117.7	80.7	50.9	116.4	91.9	58.2	218.5
0–4–7	84.9	81.0	123.0	76.0	105.0	77.6	46.7	120.8	84.2	52.6	212.9
0–8–7	84.1	80.9	126.5	71.2	115.4	79.8	54.4	132.3	86.4	54.1	221.3
0–12–7	86.0	84.5	129.2	73.7	120.8	79.4	53.0	139.0	91.6	59.9	229.3
0–16–7	86.2	78.9	128.3	77.6	130.7	81.5	52.8	126.4	98.4	55.5	229.1
Av. for the four phosphorus treatments	85.3	81.3	127.0	74.6	118.0	79.6	51.7	129.6	90.2	55.5	223.1

TABLE 5

Influence of phosphorus on the percentages of nitrogen in dry alfalfa hay, 1935–1938

FERTILIZER TREATMENT	1935		1936		1937			1938			ANNUAL AVERAGE
	Cuttings										
	First	Second	First	Second	First	Second	Third	First	Second	Third	
Check (no phosphorus).....	2.47	2.94	2.64	2.86	2.83	2.73	2.99	2.44	2.95	2.44	2.73
Av. for the four phosphorus treatments.	2.51	2.95	2.79	2.85	2.95	2.80	2.90	2.63	2.88	2.49	2.77

treatment. With the exception of the crop for 1937, the percentage of nitrogen is higher in hay from the second cutting than from either of the other cuttings. As hay was comparatively free from weeds and grass, the variations can hardly be attributed to impurities in the hay.

PHOSPHORUS CONTENT OF THE SOIL

The phosphorus content of this soil in its normal condition is not above the average for this type. Unfortunately, samples were not taken from the different plots when the work was started in 1924. Samples were taken in

1932, 1935, and 1937 and were analyzed for phosphoric acid (P_2O_5). The results are shown in table 6. The average percentages of P_2O_5 for the four check plots are slightly higher than those for the two plots that receive the low phosphorus treatment, 0–4–7. Since the yields were virtually the same (see table 1), there seems no good reason for this. Beginning with the phosphorus treatment, the phosphorus content of the soil increases slightly with increase in the amount of phosphorus supplied. There is, however, no significant change in the phosphorus content for the particular treatments over the 5-year period.

TABLE 6

Percentage of P_2O_5 in air-dry soil from plots receiving varying phosphorus treatments

FERTILIZER TREATMENT	SURFACE SOILS			SUBSURFACE SOIL
	1932	1935	1937	1937
0–0–7	0.138	0.129	0.127	0.085
0–4–7	0.119	0.125	0.116	0.072
0–8–7	0.140	0.149	0.146	0.074
0–12–7	0.159	0.158	0.166	0.087
0–16–7	0.173	0.162	0.177	0.068

TABLE 7

pH, total P_2O_5 content, and total nitrogen content of soil profiles, 1938 samples

(Air-dry basis)

DEPTH	PLOT 6—0–16–7			PLOT 7—0–0–7		
	pH	P_2O_5	N	pH	P_2O_5	N
inches		*per cent*	*per cent*		*per cent*	*per cent*
0–6	6.05	0.171	0.075	6.28	0.140	0.085
6–12	6.50	0.107	0.057	6.40	0.089	0.059
12–18	6.60	0.077	0.034	6.65	0.050	0.026
18–24	6.55	0.086	0.030	6.65	0.050	0.025
24–30	6.65	0.073	0.024	6.55	0.060	0.018
30–36	6.85	0.069	0.025	5.60	0.080	0.017
36–42	6.88	0.086	0.016	6.25	0.096	0.016

In an effort to explain the failure of the alfalfa to show a response to applications of phosporus, the phosphorus content of the soil profile was studied. Soil samples were taken from two of the plots (plot 6, 0–16–7 treatment, and plot 7, 0–0–7 treatment) at 6-inch intervals to a depth of about 42 inches. In taking these samples the roots of alfalfa plants were followed to a depth of more than 42 inches where they broke off and could not easily be followed further.

Table 7 shows the pH values, total P_2O_5 content, and total nitrogen content of the 6-inch sections of soil from the two plots. It will be noted that the pH tends to increase with depth, though there is slight exception to this in

the lower depths of the plot 7 profile. In plot 6, the P_2O_5 decreases to the 18-inch depth and thereafter is somewhat irregular; in plot 7, it decreases to the 18-inch depth, and then tends to increase to the 42-inch depth. The low P_2O_5 content at the 18- to 30-inch level of this plot is not accounted for unless it be that the roots have especially depleted the supply of phosphorus in this zone. The average percentage of P_2O_5 for the plot 6 profiie is 0.096, and that for the plot 7 profile, 0.081. If the weight of 1 acre of soil to the depth of 42 inches is placed at 13,000,000 pounds, the total P_2O_5 content is approximately 10,000 to 12,500 pounds. Here is an enormous reservoir of phosphorus for the alfalfa roots to draw upon. Probably most of the roots extend to a greater depth than 42 inches and thus have access to an even larger supply of phosphorus.

The 1932 surface samples were examined for available phosphorus by the Truog method (8), 0.002 *N* H_2SO_4, and by the LaMotte-Truog method, but no very definite relationship was found between the available phosphorus and the phosphorus treatment of the soil. For the Truog method, the results varied from about 200 to over 350 p.p.m. By the LaMotte-Truog test, the variation was from 125 to 320 p.p.m.

Although it is certain that a large percentage of the total phosphorus is in a slowly available form, it is not at all unlikely that with such an enormous reserve there would be an abundance of available phosphorus to supply for a good many years the 13 or 14 pounds of P_2O_5 required for each ton of alfalfa hay.

Truog (9) has directed attention to a case somewhat similar to the one described here.

The results set forth in this paper must not be taken as the endorsement of a policy of using no phosphorus for alfalfa; they show what happened under the conditions described for a period of 4 years only. Under other conditions and over a longer period of time, the results might well be different. The work indicates a need for a more careful study of the mineral requirements of alfalfa and also of the nutrient-supplying power of the soil. If, by proper management, good crops of alfalfa can be grown continuously for 4 years, there seems no good reason why they may not be grown for 8 or even 12 years if we can learn more of the nutrient requirements and also learn more about overcoming the adverse conditions which may quickly unbalance the best of cultural and fertilizer practices.

SUMMARY

Alfalfa was grown for 4 years, 1935–1938 inclusive, on $\frac{1}{44}$-acre plots that had received no phosphorus treatment since 1924, and also on plots that received varying amounts of phosphorus annually. No nitrogen was applied, except a small amount at the time of seeding; and potash was applied uniformly to all plots.

The 4-year average gave virtually the same yield for the check plots as for those that received the highest amount of phosphorus.

Analysis of the hay showed a slight tendency toward a higher percentage of phosphorus in the hay with increase in the amount of phosphorus applied. The percentages of nitrogen, ash, CaO, and MgO in the hay were not significantly influenced by the amount of phosphorus applied. The percentage of CaO in the hay was five to seven times as high as the percentage of MgO.

Analysis of the soil taken from the various plots in 1932, 1935, and 1937 shows a tendency toward a slight increase in the total phosphorus content with increase in the amount of phosphorus applied, beginning with the lowest application.

Analysis of profile samples from two of the plots, No. 6, which received the maximum phosphorus application, and No. 7, which received no phosphorus, shows slightly more phosphorus in the 6-inch layers down to 30 inches for plot 6 than for plot 7.

Calculation of the total phosphorus in the 42-inch layer indicates a reservoir of phosphorus amounting to 10,000 to 12,500 pounds P_2O_5 to the acre. As an explanation for the failure of alfalfa to show a response to phosphorus treatment in this case, it is suggested that the plants may find here enough available phosphorus to produce a maximum crop.

The results reported here are for the conditions under which the experiment was conducted and are not to be taken as endorsing a policy of omitting phosphorus from alfalfa fertilizers. Further work along this line is in progress.

REFERENCES

(1) Ames, J. W., and Boltz, G. E. 1912 Nitrogen and mineral constituents of the alfalfa plant. Ohio Agr. Exp. Sta. Bul. 247.

(2) Archibald, J. G., Nelson, P. R., and Bennett, E. 1932 A three-year study of the chemical composition of grass from plots fertilized and grazed intensively. *Jour. Agr. Res.* 45: 627–640.

(3) Blair, A. W., and Prince, A. L. 1932 The influence of phosphates on the phosphoric acid content of the plant. *Jour. Agr. Res.* 44: 579–590.

(4) Blair, A. W., and Prince, A. L. 1938 Potash saves alfalfa, clover and soybeans. *Better Crops with Plant Food.* 22 (8): 6–8.

(5) Brown, B. A. 1932 The effects of fertilization on the chemical composition of vegetation in pastures. *Jour. Amer. Soc. Agron.* 24: 129–145.

(6) Forbes, E. B., Whittier, A. C., and Collison, R. C. 1910 The mineral nutrients in blue-grass. *Ohio Agr. Exp. Sta. Bul.* 222: 39–53.

(7) Lipman, J. G., and Blair, A. W. 1917 Influence of lime on the yield of dry matter and nitrogen content of alfalfa. N. J. Agr. Exp. Sta. Bul. 316.

(8) Truog, E. 1930 The determination of the readily available phosphorus of soils. *Jour. Amer. Soc. Agron.* 22: 874.

(9) Truog, E. 1938 A modern conception of soils, fertilizer action, and plant nutrition. *Proc. Fourteenth Ann. Conv. Natl. Fert. Assoc.* 1938: 76.

(10) Van Slyke, L. L. 1912 Fertilizers and Crops. Orange Judd Co.

RATES OF TEMPERATURE CHANGES IN SOILS AT VARIOUS MOISTURE CONTENTS[1]

H. A. WADSWORTH[2]

Hawaii Agricultural Experiment Station

Received for publication November 26, 1938

In a recent paper[3] attention was directed to the effect of temperature of the environment upon the heat of wetting of two widely differing soils. In this work it was indicated that, with a given moisture content, high values for the heat of wetting were obtained when the determination was made at a low temperature, and conversely.

If the heat of wetting of a soil be interpreted as an expression of the kinetic energy lost by water when it is adsorbed by the colloidal material upon saturation, it follows that low values for the heat of wetting should be associated with soil—soil-moisture systems close to their capacity for this type of adsorption. Cooling a soil must result in a decrease in the kinetic energy in the system, and this reduction of kinetic energy in the cooled system must be rectified during the process of saturation, with the abnormally high heat of wetting results which have been noted.

From this reasoning and the converse argument from soils at higher temperatures, it would appear that significant modifications in the energy relations in a soil—soil-moisture system take place during temperature changes. For example, with the cooling of a moist soil it would appear that the reduction of kinetic energy in the moisture within the soil would be associated with the evolution of heat, and the warming of a cold soil would be associated with an absorption of heat without increase in temperature. Such thermal characteristics of relatively dry soils do not easily lend themselves to quantitative determination, but qualitative determinations are simple and convincing.

EQUIPMENT AND PROCEDURE

The well-known law of rates of cooling and heating permits such a qualitative demonstration. Thus, if an inert, homogeneous body at an initial temperature θ_1 be placed in a constant temperature bath of temperature θ_2,

[1] Published with the consent of the acting director of the Hawaii Agricultural Experiment Station.

[2] Irrigation engineer.

[3] Wadsworth, H. A. 1939 A study of some factors influencing the heat of wetting of soils. *Soil Sci.* 47: 385–390.

the reduction or increase of temperature of the body will follow a well-defined exponential law. In usual terms we have,

$$\theta_t = \theta_0 \epsilon^{-at} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (A)$$

where θ_0 = original excess or deficiency of temperature of a body over its surrounding

θ_t = the instantaneous excess or deficiency of temperature at the end of time t

a = a constant depending upon the thermal characteristics of the body.

It may be assumed that if the experimental set-up is adequate, any departures from the relationships shown in equation A must be due to thermal abnormalities in the material under study. Thus, if heat is actually evolved during the process of cooling, a hot soil in a cold external bath might show a departure from the relationship in A in the sense that the fall of temperature in the soil would be at a slower rate than the law required. Similarly, with a warming soil the rate of soil temperature increase should be slower than the law specifies if heat is actually adsorbed without increase of temperature under such conditions.

Equation A evidently lends itself well to such studies, since the logarithmic form is a stright line:

$$\log_{10} \theta_t = K - a_1 t \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B)$$

where K and a_1 are consolidated constants resulting from the evident manipulation. Thus, if the logarithm of the temperature difference at any time t is plotted against t, a straight line should result if the simple law is followed. Any departures from such a rectilinear arrangement may be considered to be the result of internal thermal effects.

In the present study the material was placed in a thin-walled pyrex test tube, fitted with a tight cork and a certified themometer, reading to 0.05°C. This assembly was brought to constant temperature in a water bath and allowed to stand at the temperature of the bath for 1 hour At the end of this period the tube was quickly transferred to a calorimeter carrying water at a desired temperature. In view of the adequate insulation of the calorimeter and the large amount of water used in it, no measurable change in the temperature of the bath was noted during the time of observations. The calorimeter was equipped with an accurate thermometer; stirring facilities were provided.

During the actual procedure, zero hour was established on a stop watch at the instant the tube was introduced to the water bath. Observations were made upon each of the thermometers at minute intervals. The difference (θ_t) was noted in each case and the logarithm plotted against the corresponding time t.

RESULTS

Inert materials such as water and oven-dry silica sand were used to test the application of the law to the present problem and to give evidence of the adequacy of the equipment. Results are shown in figure 1.

A second series of tests was devoted to a preliminary study of Ewa soil which has already been described.[4] One sample was freshly oven dried, another was wetted to maximum field capacity by the suction method of Bouyoucos.[5] Results in figure 2 indicate that the Ewa soil, at these extremes of moisture content, follows the familiar law of thermal transfer. A similar test on oven-dry Superior clay loam shows the same relationship (fig. 2).

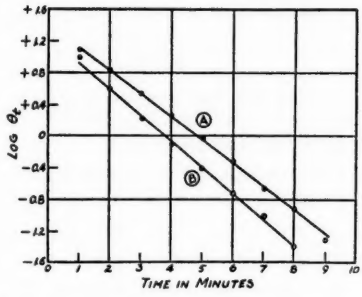


FIG. 1. RATE OF TEMPERATURE CHANGE FOR INERT MATERIALS INITIALLY COLD: *A*, DRY SILICA SAND; *B*, DISTILLED WATER

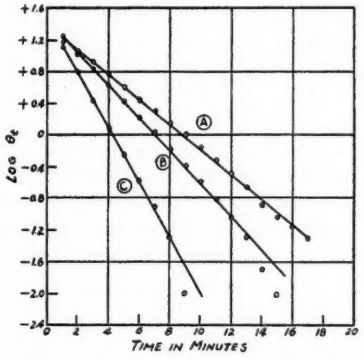


FIG. 2. RATE OF TEMPERATURE CHANGE FOR SOILS: *A*, EWA SOIL, OVEN DRY, WARMING CURVE; *B*, SUPERIOR CLAY LOAM, OVEN DRY, WARMING CURVE; *C*, EWA SOIL AT MAXIMUM FIELD CAPACITY, WARMING CURVE

Abnormalities appear when soils at moisture contents between these limits are used. When the Ewa soil, carrying about 8 per cent moisture, was warmed to 40°C. and placed in a calorimeter containing water at room temperature, the rate of fall of temperature within the soil was much lower than would be expected from figures 1 and 2. Moreover, no straight line was obtained. A similar form of curve resulted when Ewa soil with 8 per cent moisture was cooled to about 10°C. and then warmed by thermal transfer in a calorimeter at room temperature. When the Ewa soil carried a moisture content of

[4] Wadsworth, H. A. 1938 Some thermal phenomena in a selected Hawaiian soil. *Soil Sci.* 45: 251–262.

[5] Bouyoucos, G. J. 1929 A new simple and rapid method for determining the moisture equivalent of soils and the role of soil colloids on this moisture equivalent. *Soil Sci.* 27: 233–240.

about 15 per cent, the same general effect was noted. These series are plotted in figure 3.

Similar results were obtained with the Superior clay loam. Figure 4 gives the warming and cooling histories for this soil at 5 per cent moisture content.

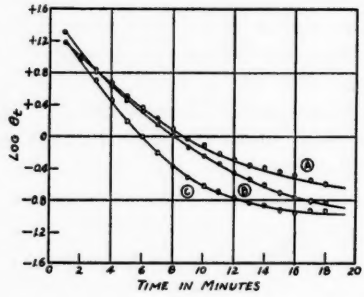


FIG. 3

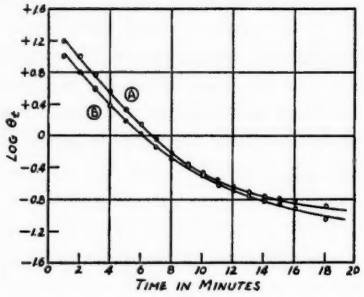


FIG. 4

FIG. 3. RATE OF CHANGE OF TEMPERATURE OF MOIST SOILS: *A*, EWA SOIL, COOLING CURVE, AT 8 PER CENT MOISTURE; *B*, EWA SOIL, WARMING CURVE, AT 8 PER CENT MOISTURE; *C*, EWA SOIL, WARMING CURVE, AT 15 PER CENT MOISTURE

FIG. 4. RATE OF CHANGE OF TEMPERATURE OF SUPERIOR CLAY LOAM AT 8 PER CENT MOISTURE: *A*, WARMING CURVE; *B*, COOLING CURVE

DISCUSSION

The similarity of all the curves obtained with soils carrying moisture percentages between oven-dryness and a point somewhat below maximum field capacity draws attention to an energy relationship which, as far as is known, has escaped reference in the literature. The simplicity of the technic and the basic precision in the observations, as evidenced by the smoothness of the resulting curves, add emphasis to the belief that the observed phenomenon is a reflection of an inherent soil characteristic.

It is to be noted that the ordinates are functions of temperature difference and are not readily translated into measures of heat But in all instances of soils at moderate moisture contents, the rate of change of this temperature difference is less than that noted when the soils are absolutely dry or when they are wetted above a critical, and as yet unidentified, moisture content.

The two cases—that is, a cooling soil and a warming soil—present two different aspects of the same general conception.

In the cooling soil we have a definite thermal gradient in favor of a flow of heat from the soil to the constant temperature reservoir. The loss in heat from the soil, which must result from such a gradient, is reflected in a decrease of thermometer reading, but apparently the fall of temperature is not governed solely by the accepted law of cooling. In all cases the thermometer reading falls at a rate slower than simple theory demands. One evident explanation lies in the assumption that the process of cooling of the soil, *per se*, results in

the evolution of heat. If this assumption be made, it is evident that the thermometer integrates two effects: One of these is the loss of heat in view of the thermal gradient; the other is the gain of heat resulting from cooling. In the conventional example of an inert, homogeneous body there is no gain of heat by cooling; consequently, any manifestation of such a source of heat in a more complex system must reduce the rate of temperature fall.

In the warming soil, the situation is reversed, but the observable results in temperature change are similar. In this case the thermal gradient is toward the soil, and heat flows into the tube. With an inert material the rate of temperature increase would be determined by the law of heating or cooling. But another factor complicates the picture when a relatively dry soil is used. This appears to be an absorption of heat without change of temperature. Consequently some of the heat flowing into the system under the influence of the thermal gradient finds no expression in temperature increase, and the observed rate of increase of temperature is slower than would be expected from the simple law.

The fact that these unexpected thermal effects are evident within only a limited range of soil moisture contents adds credence to the belief that moisture between oven dryness and maximum field capacity may be divided between two categories, each of which is governed by its own physical relationships. Although this demarcation, if it exists, cannot be drawn from the present work, it seems clear that the phenomenon of "heat of cooling" and the converse relationship disappear at a moisture content close to the permanent-wilting percentage. It will be recalled that no significant heat of wetting is apparent at moisture contents higher than the permanent-wilting percentage.[6]

From the present work, no defendable conclusion can be reached, with respect to the thermodynamical reason for the phenomena reported. But this unsuspected property of soils is reported at this time, since it may open the way to a new approach to studies of soil moisture and surface-energy relationships. The technic is simple, and precise results can be readily obtained.

SUMMARY

Some evidence is offered to support the assumption that the simple cooling of a soil generates heat.

When cold soils are warmed, heat is absorbed without a corresponding temperature increase.

Although the moisture limits within which these phenomena are apparent have not as yet been defined, there is some evidence that the percentage of moisture within the soil must lie between the maximum field capacity and a very low moisture content if the effect is to be noted.

The problem was attacked through a study of rates of temperature change and a comparison with the classic law of cooling.

[6] See footnote 3.

EFFECT OF TEMPERATURE UPON THE MOISTURE-CONTENT—SURFACE-FORCE CURVE OF SOIL[1]

H. A. WADSWORTH[2]

Hawaii Agricultural Experiment Station

Received for publication December 27, 1938

An expression of the relationship existing between the moisture content of a soil and some function of the force with which that moisture is held in the soil is of practical value as well as scientific interest. In early work, Shull (12) used curves expressing this relation to demonstrate the physical basis for the wilting of plants. More recent work with better methods by Puri et al. (9), Thomas (13, 14), Edlefsen (5), Alexander and Haring (1), and others has demonstrated the validity of Shull's general conclusions as to the shape of the curve, although a question has been raised with respect to its detailed interpretation. The argument of the capillary potential (6) centers about the same relationship, and Schofield's (10) newly introduced pF conception is but a simplification of expression for results obtained in attempts to measure the force with which moisture at a specific percentage is held to the soil particle.

From a composite picture of the work done in the field, one is left with the impression that the entire range of moisture contents from oven dryness to maximum field capacity, or saturation (8), can be expressed by a smooth but not mathematically simple curve. Moreover, one gains the idea that any soil can be well described by a single curve [or by a double curve (7), if both the wetting and drying arms of the so-called hysteresis loop are described] which is specific for the soil. Fom such a curve one can, supposedly, determine the soil-moisture percentage in equilibrium with water vapor over 3.3 per cent sulfuric acid, or other standard concentration, the permanent-wilting percentage, the moisture equivalent (11), or the moisture content which would be associated with any specified freezing-point depression (10).

Except for recent work, the common procedure has been to determine soil-moisture percentages which are in equilibrium with specified relative humidities established by sulfuric acid solutions in proper concentrations. From such figures the corresponding values for surface force can be obtained by acceptable relations from thermodynamics. Early work by Bouyoucos (3) and more recent work by Schofield (10) suggest that the freezing-point de-

[1] Published with the consent of the acting director, Hawaii Agricultural Experiment Station.

[2] Irrigation engineer.

pression for a soil might be used to supply additional points for such curves. It is interesting to note that the vapor pressure methods become increasingly difficult (8) at about the same point at which observations upon the freezing-point depression become practicable.

THE NEED AND EFFECT OF TEMPERATURE CONTROL

It is evident that temperature control is necessary if vapor pressures are to be translated into terms of relative humidities. And in most, if not all, cases the temperatures at which equilibrium has been attained have been carefully specified. The fact remains, however, that working at a particular temperature creates a special case. It is doubtful, perhaps, whether or not small differences in the temperature are important in changing the location of the curve within its zone of greatest usefulness, which is near the moisture percentage associated with the permanent wilting of plants. But at lower percentages the possibility of modification with temperature appears to be highly significant and of considerable theoretical importance.

Baver and Winterkorn (2) report that the water-adsorption capacity of modified soil materials in a constantly maintained humidity of 94 per cent decreases rapidly as the temperature is increased. Puri, Crowther, and Keen (9) in an earlier work report the same effect with three soils of widely differing mechanical analysis. These English workers, however, report and find significance in differences which are relatively small compared with those reported by the American investigators. On the other hand Veihmeyer and his colleagues (15) show that the moisture equivalent for each of the soils upon which they report is increased by increases in temperature during the period of saturation prior to the determination. A similar result was obtained in some unreported work in Hawaii with a local soil. Although such scattered observations are inadequate for the formation of a dependable conclusion, they do give some evidence that temperature effects may be marked in modifying the shape and position of the moisture-content—surface-force curve if drawn in the conventional form with relative humidity on the vertical axis and moisture percentage on the horizontal axis. From the limited data available it would appear that increases in temperature move the curve to the left if the corresponding relative humidity is less than a certain maximum, and to the right if that maximum is exceeded.

The present work was designed to verify the conclusions of earlier workers, with soils which are entirely different from the materials previously used, and to search for any tendency for such moisture-content—surface-force curves to cross when drawn at different temperatures.

EQUIPMENT, METHODS, AND RESULTS

In this work a return was made to the early methods of Shull (12) in which saturated solutions of inorganic salts were used to establish and maintain

specified humidities. International Critical Tables and supporting references give the humidities established by many salts at different temperatures.

Continuous agitation of surplus reagents for long periods in the reservoirs of good 8-inch desiccators provided assurance that saturation had been effected. In every case a supply of undissolved salt remained in the bottom of the desiccator after such treatment.

Two soils were used: lateritic soil from the Ewa district of Oahu, already described (16), and Superior clay loam. Since both the drying arm and the wetting arm of the curves are of interest, some samples of each soil were oven-dried as a preliminary to introduction to the desiccators, and others were wetted to maximum field capacities by the suction method (4). After such preliminary treatments samples were weighed into small, tared aluminum boxes and admitted to the desiccators. Determinations were made in duplicate. Thus each desiccator carried eight boxes. Since tiering was necessary to permit the packing of eight boxes in the available space, a reversal of packing was effected at each opening of the desiccators.

Three temperatures were maintained. One of these was the temperature of the tap water in the laboratory, which under conditions of steady flow held a temperature close to 25.6°C. throughout the run. Another series was held at 40°C. in a water bath with conventional temperature control. In this case losses of water by evaporation were automatically replaced from an inverted flask. The third series was stored in an electric refrigerator. Here the temperature control was not so effective as in the other two cases. Frequent observations upon this temperature gave a value of about 8°C.

In the hot series and the room temperature series desiccators were completely submerged in their water baths to avoid temperature gradients within the air space above the surfaces of the solutions. Since the desiccators tended to float, lead masses were placed on the covers. It is evident that great care is necessary if this procedure is used. A generous use of sealing material and a careful rubbing out of all air bubbles between the desiccator and its cover are essential.

Desiccators were opened as infrequently as possible. Within 6 weeks, equilibrium weight had been reached by all samples. Equilibrium was said to exist when a weighing box lost less than 0.5 mgm., on a 3-gm. sample, within 1 week. Samples were then dried in a standard oven at 110°C., and the moisture contents were determined as usual. The duplicate samples provided acceptable checks. The greatest difference lay in the two samples on the drying arm in the cold series of the Ewa soil at the highest humidity. Here the difference between the duplicates was 0.79 per cent moisture. The other duplicates, particularly in the lower humidities, provided much better checks.

Results in simplified form are given in table 1. The moisture percentage given is the average of the duplicates.

The data in table 1 are given in graphic form in figure 1. Although such curves, drawn through but three points, do not lend themselves to detailed

TABLE 1

Soil moisture in equilibrium with varying humidities at varying temperatures

RELATIVE HUMIDITY	EWA SOIL		SUPERIOR CLAY LOAM		SALT
	Moisture		Moisture		
	Drying arm	Wetting arm	Drying arm	Wetting arm	
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
			Hot series (40.0°C.)		
96.3	17.06	16.14	6.02	4.68	K_2SO_4
89.0	9.70	8.33	4.13	3.05	KNO_3
74.7	5.09	4.24	3.06	2.46	NaCl
			Room temperature (25.6°C.)		
97.1	19.00	16.82	7.08	5.24	K_2SO_4
93.5	15.54	12.13	4.96	3.88	KNO_3
62.0	4.68	3.34	2.94	2.27	NH_4NO_3
			Cold series (8°C.)		
97.9	20.20	18.36	12.02	7.04	K_2SO_4
86.6	15.36	11.76	5.12	3.96	KCl
75.3	7.50	5.66	3.97	3.04	NaCl

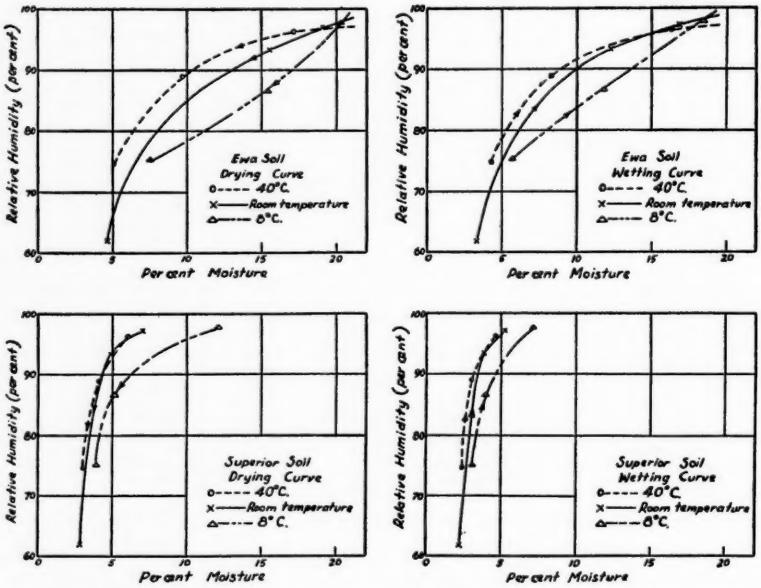


FIG. 1. RELATIVE-HUMIDITY—MOISTURE-CONTENT CURVES OF SOILS AT VARIOUS TEMPERATURES: HOT REGIME, 40°C.; ROOM TEMPERATURE, 25°C.; COLD, 8°C.

quantitative interpretations, there is considerable evidence that temperature is highly important in determining the position of the moisture-content—surface-force curve. The curves for the Ewa soil give promise of coincidence at humidites lower than the limit of the present work and, by extrapolation, give some evidence of crossing somewhere in the higher humidities, as has been suggested.

The relationship is not so clear with Superior clay loam. When this soil is used, a reduction of temperature below room temperature appears to be much more significant in locating the curve than is an increase above room temperature. Moreover, there seems to be no marked evidence that the curves for the higher temperatures would cross the curve obtained from the cold series.

No explanation can be given for the unexpected shape of the "cold" curve with the Ewa soil. Here, on both the wetting and drying arms, the curves lose their characteristic convex shape. In view of the similarity of the moisture percentages for the duplicate results, it seems probable that this effect is not due to error.

It should be noted that the two soils differ in several important respects. Ewa soil is lateritic, composed largely of particle sizes within the silt and clay groups, and is high in weathered iron and aluminum compounds. The Superior soil is relatively coarse-grained and carries a clay separate relatively rich in compounds of silica.

SUMMARY

Some evidence is presented to support the belief that the position of the surface-force—moisture-content curve over a considerable range depends upon the temperature at which the relations are noted.

In general, a lowering of the temperature at which the determination is made, within certain limits, increases the moisture content of a soil in equilibrium with a specified relative humidity.

With one of the soils studied there is some evidence that the curves cross at a relatively high humidity.

With Superior clay loam a reduction of the temperature below normal room temperature seems to be more effective in shifting the curve than is a corresponding increase above room temperature.

REFERENCES

(1) Alexander, L. T., and Haring, M. M. 1936 Vapor pressure-water content relations for certain typical soil colloids. *Jour. Phys. Chem.* 40: 195–205.

(2) Baver, L. D., and Winterkorn, H. 1935 Sorption of liquids by soil colloids: II. Surface behavior in the hydration of clays. *Soil Sci.* 40: 403–419.

(3) Bouyoucos, G. J. 1916 Further studies on the freezing point lowering of soil. Mich. Agr. Exp. Sta. Tech. Bul. 31.

(4) Bouyoucos, G. J. 1929 A new and rapid method for determining the moisture equivalent of soil and the role of soil colloids on the moisture equivalent. *Soil Sci.* 27: 233–240.

(5) EDLEFSEN, N. E. 1934 A new method of measuring the aqueous vapor pressure of soils. *Soil Sci.* 38: 29–35.
(6) GARDNER, W., et al. 1922 The capillary potential function and its relation to irrigation practice. *Phys. Rev.* 20: 196.
(7) KEEN, B. A. 1931 The Physical Properties of the Soil. Longmans Green and Co., New York.
(8) PURI, A. N. 1925 A critical study of the hygroscopic coefficient of soils. *Soil Sci.* 15: 272–283.
(9) PURI, A. N., CROWTHER, E. M., AND KEEN, B. A. 1925 The relation between the vapour pressure and water content of soils. *Jour. Agr. Sci.* 15: 68–88.
(10) SCHOFIELD, R. K. 1935 The pF of the water in the soil. *Trans. Third Internatl. Cong. Soil Sci.* 2: 38–48.
(11) SCHOFIELD, R. K., AND DA COSTA, B. 1935 The determination of the pF at permanent wilting and at moisture equivalent by the freezing point method. *Trans. Third Internatl. Cong. Soil Sci.* 2: 6–10.
(12) SHULL, C. A. 1916 Measurement of surface forces in soils. *Bot. Gaz.* 62: 1–29.
(13) THOMAS, M. D. 1921 Aqueous vapor pressure of soils. *Soil Sci.* 9: 409–434.
(14) THOMAS, M. D. 1924 Aqueous vapor pressure in soils: II. Studies in dry soils. *Soil Sci.* 17: 1–18.
(15) VEIHMEYER, F. J., OSERHOWSKY, J., AND TESTER, K. B. 1927 Some factors affecting the moisture equivalents of soils. *Proc. and Papers First Internatl. Cong. Soil Sci.* 1: 1–23.
(16) WADSWORTH, H. A. 1938 Some thermal phenomena in a selected Hawaiian soil. *Soil Sci.* 45: 251–262.

STUDIES IN ELECTRODIALYSIS OF SOILS: IV. EFFECT OF TEMPERATURE, pH VALUE, AND DEGREE OF ALKALIZATION

AMAR NATH PURI, R. C. HOON, AND C. L. DHAWAN

Irrigation Research Institute, Lahore, India

Received for publication December 14, 1937

It has been shown (5) that the rate of electrodialysis of soils depends to some extent on the nature of the replaceable base. This was ascribed to the differences in the ionization of the various bases on the surface. It is also well known that the amount of alkali coming out gradually decreases as the electrodialysis proceeds. Obviously, therefore, the soil reaction must play an important part in controlling the speed of electrodialysis. The difference in the speed of electrodialysis of Ca and Na ions also suggested the possibility of separating these ions from soils of varying degree of alkalization. The object of this investigation was to gather exact information on these points.

EXPERIMENTAL

Electrodialysis was conducted in the cell with the rotating cathode previously described (3). Soils of varying pH value and degree of alkalization were prepared from the H-soil by neutralization with appropriate amounts of various alkalies or mixtures of NaOH and $Ca(OH)_2$. In preparing soils at different pH values, the amount of alkali was kept constant, the amount of soil being varied. Current was kept at 0.1 ampere throughout the experiment, and the alkali displaced was titrated every half hour. The results are graphically represented in figure 1, the total alkali recovered being plotted against successive time intervals in every case. It is seen that the rate of electrodialysis increases with the increase in pH value. This increase in rate, however, is generally confined to the first half hour, after which the various curves show virtually the same relative shift at different time intervals. This is easily comprehensible when it is remembered that the pH value decreases as the electrodialysis proceeds, so that the rate of recovery tends to equalize in time.

The difference in the rate of recovery of the various ions is well brought out by these curves. This suggested the possibility of separating exchangeable bases by electrodialysis. Separation of metals by electrodialysis using the principle of graded potential is well known. An application of the same principle is the polarographic method of analysis. Polarographic current-voltage curves have been discussed before (4). The application of the same principle to the quantitative separation of exchangeable bases is rather limited, because low voltages required for such separations are not possible in the case of soils

on account of the extremely low conductivity of soil suspensions, which can admit currents of the order of a few microamperes under these circumstances. There is, however, the possibility that at low current densities a separation of the monovalent and divalent bases might be effected. It was felt that even if the separation were not complete, a knowledge of the magnitude of the differences would be of great interest in dealing with soils having varying ratios of exchangeable Na/Ca.

Degree of alkalization is defined as the percentage of exchangeable Na on the total exchangeable bases. Its utility in characterizing alkali soils has been discussed in a previous publication (1). In discussion of the behavior of such soils elsewhere (2), the hypothesis was put forward that the toxicity of alkali

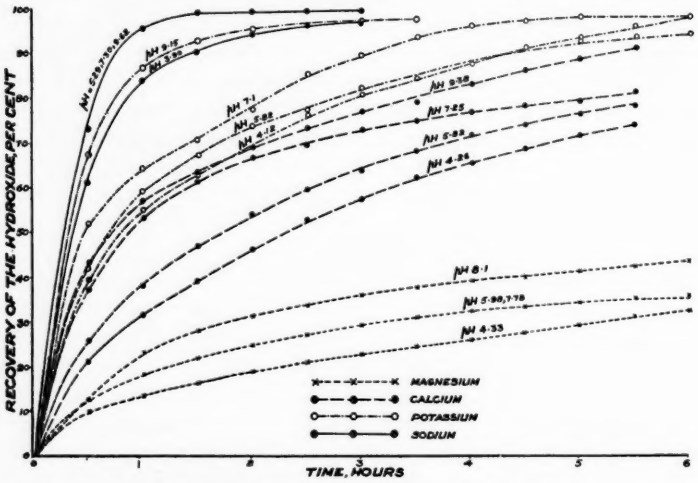


FIG. 1. EFFECT OF pH ON THE RATE OF RECOVERY OF BASES BY ELECTRODIALYSIS

soils was largely due to the deficiency of Ca under highly alkaline conditions. It was felt that the electrodialysis of such soils might throw light on the availability of Ca in them. As a preliminary to this study, the rate of electrodialysis of a Na-soil was determined by the use of low currents. It was found that 66 to 70 per cent of the total exchangeable Na could be removed in 19 hours with a current of 0.005 ampere. The use of lower currents was not considered feasible. Since electrodialysis at low current densities is likely to spread over a long period, involving a large volume of the electrodialyzate and necessitating several titrations, the alternative method of estimating Na gravimetrically was also considered. A comparison with the values obtained by simple titrations as hydroxide, showed that the gravimetric method gave essentially similar results, and as the soils contained no bases other than those under considera-

tion, simple titrations of the electrodialyzate furnished all the information required in this connection.

Two series of soils, one at pH 7 and the other at pH 9, of varying degrees of alkalization were prepared for this study. Current was maintained at 0.005 ampere in every case throughout the experiment, which lasted for 33 hours. The soil was first freed from exchangeable bases by treatment with 0.05 *N* HCl and then shaken for 48 hours with varying ratios of $NaOH/Ca(OH)_2$, the total quantity of alkali being kept constant. In the two series, 52 and 78 m.e. of total alkali per 100 gm. of soil were used to obtain soils at pH 7 and pH 9 respectively. These quantities were determined from the titration curves of the soil. The final pH values differed slightly from 7 and 9, but the variations

TABLE 1

Recovery of bases by electrodialysis of (Na + Ca)-soils at different degrees of alkalization, at pH 7, and with current density of 0.005 ampere

DEGREE OF ALKALIZATION...*per cent*	15.40		23.07		30.77		38.46		53.84		69.23		84.61	
Time intervals	Na	Ca	Na	Ca	Na	Ca	Na	Ca	Na	Ca	Na	Ca	Na	Ca
hours	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
½	2.19	1.20	1.53	0.70	2.08	0.80	1.16	0.60	2.72	0.40	1.98	0.60	3.01	....
1	0.44	1.10	1.79	0.90	2.31	0.70	1.71	0.60	2.19	0.60	2.10	0.40	3.21	....
1½	....	1.10	....	1.10	1.12	0.80	1.20	0.80	2.52	0.60	2.27	0.30	3.16	0.20
2	....	1.00	....	1.20	0.20	0.90	1.76	0.60	0.88	0.50	1.60	0.70	1.90	0.20
2½	....	1.00	....	1.00	0.18	1.00	0.65	0.60	1.12	0.30	1.51	0.40	2.05	0.40
3	....	0.70	....	0.80	....	1.20	1.05	0.70	0.34	0.60	1.30	0.50	1.41	0.20
3½	....	0.80	....	1.00	....	1.30	0.30	0.90	0.21	0.50	0.85	0.40	2.12	0.30
4	....	0.90	....	0.90	....	1.00	....	1.10	....	0.70	0.65	0.20	2.19	0.10
4½	....	0.90	....	0.60	....	0.80	....	1.00	....	0.40	0.78	0.10	2.20	0.20
5	....	0.80	....	0.80	....	0.80	....	0.90	....	0.70	0.40	0.40	1.78	0.30
5½	....	0.70	....	0.90	....	0.60	....	1.00	....	0.60	0.18	0.80	0.80	0.50
Total.....	2.63	10.20	3.32	9.90	5.89	9.90	7.83	8.80	9.98	5.90	13.62	4.80	23.83	2.40

were not considered significant for the purpose of this experiment. The results are given in tables 1 and 2. It will be seen that as the degree of alkalization increases, the amount of Na in the electrodialyzate increases, and the amount of Ca decreases.

The practical significance of these values will be clear from figure 2, in which the ratio Ca/Na in the entire electrodialyzate is plotted against degree of alkalization. The two curves are essentially similar, but at the higher pH values probably the toxic ratio is reached at a lower degree of alkalization than at the lower pH value. How the deficiency of Ca in soils of high alkalinity, which is reflected in the low Ca/Na ratio in the electrodialyzate, may affect the crop yield is brought out by the curve showing the relation between degree of alkalization and yield of wheat. The observations on yield trials refer to

a reclamation farm (Montgomery) having a soil quite different from the one used for electrodialysis. The striking similarity of the curves emphasises the fact that the fundamental cause of infertility in alkali soils lies in the deficiency of available Ca. It is not unlikely that electrodialysis at low current densities might furnish a useful method of finding the relative availability of different ions, especially in alkali soils in which deficiency of Ca is an important limiting factor in crop yield. Electrodialysis may be taken as ordinary dialysis or diffusion of ions speeded up by electric current. The smaller the current, the nearer the process will approach ordinary diffusion and hence natural conditions. As has been mentioned, however, it is not practicable to reduce the current much below 0.005 ampere.

TABLE 2

Recovery of bases by electrodialysis of (Na + Ca)-soils at different degrees of alkalization, at pH 9, and with current density of 0.005 ampere

DEGREE OF ALKALIZATION....*per cent*	15.40		30.07		53.84		69.23		84.61	
Time intervals	Na	Ca	Na	Ca	Na	Ca	Na	Ca	Na	Ca
hours										
½	2.89	0.40	2.70	0.30	1.90	1.80	1.69	0.90	1.70	0.25
1	1.89	0.50	2.30	0.50	2.46	1.14	2.03	0.50	2.36	0.25
1½	....	1.20	2.25	0.40	1.92	1.15	2.10	1.00	2.53	0.55
2	....	1.20	1.60	0.50	2.20	0.85	2.15	0.50	2.85	0.85
2½	....	1.20	1.40	0.80	1.75	0.80	1.80	1.10	1.90	0.60
3	....	1.20	0.80	1.60	1.52	1.00	1.65	0.85	2.10	0.20
3½	....	0.90	0.75	1.00	1.55	0.70	1.53	1.00	2.00	0.25
4	....	0.90	0.36	1.20	1.60	0.70	1.93	0.60	2.20	0.25
4½	....	1.00	0.25	1.20	1.50	0.80	1.85	0.50	2.00	0.25
5	....	1.20	0.35	1.00	1.17	0.75	1.98	0.30	1.60	0.30
5½	....	1.10	0.20	1.20	1.50	0.50	1.72	0.30	2.10	0.10
Total.....	4.78	10.80	12.96	9.70	19.07	10.19	20.43	7.55	23.34	3.85

In the course of this investigation, a question of considerable interest arose with regard to the interrelation between monovalent and divalent bases in the exchange complex. If an ordinary soluble acid is neutralized partly with $Ca(OH)_2$ and partly with NaOH, the resulting mixture is exactly the same whether the one or the other alkali is used first for neutralization. It was of interest to know whether soil acidoid would behave in a similar manner. In a heterogeneous mass like the soil, it would appear at first sight not unreasonable to suppose that the base added first might be the last to come out in the electrodialyzate. On the other hand, if the exchangeable bases held on the surface are mobile, the soil would behave like any ordinary buffer mixture and there would be no distinction regardless of which alkali was added first. The speed of electrodialysis of a (Na + Ca)-soil seemed to offer a promising means of throwing light on this question.

A completely unsaturated soil was partly neutralized with NaOH and shaken for 24 hours. An equivalent amount of $Ca(OH)_2$ or $Mg(OH)_2$ was then added, followed by shaking for another 24 hours. Another set of soils, in which the order of addition of hydroxides was reversed, was similarly prepared. The final pH value of the soil was approximately 6.70 in each case. The soils were then electrodialyzed by means of a current of 0.1 ampere. The results given in table 3 show virtually no difference in the rate of electrodialysis for Na, Ca, and Mg in the two sets of experiments. The exchangeable bases, therefore, exist in dynamic equilibrium, and the soil buffer behaves in this respect like an ordinary soluble buffer mixture.

In most work on electrodialysis, no notice is taken of the temperature. It is, however, recognized that too high a temperature should be avoided because of the possible side effects. In studying the effect of various factors on the rate of electrodialysis, it is necessary to know the exact part played by tem-

TABLE 3

Recovery of bases by electrodialysis, as affected by the order of their addition to soil

TIME	SOIL PREPARED BY ADDING $Ca(OH)_2$ FOLLOWED BY NaOH		SOIL PREPARED BY ADDING NaOH FOLLOWED BY $Ca(OH)_2$		SOIL PREPARED BY ADDING $Mg(OH)_2$ FOLLOWED BY NaOH		SOIL PREPARED BY ADDING NaOH FOLLOWED BY $Mg(OH)_2$	
	Na	Ca	Na	Ca	Na	Mg	Na	Mg
hours	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1	17.42	4.08	15.86	3.64	12.40	1.80	14.56	2.44
2	3.80	3.10	3.32	2.68	3.28	1.42	3.00	2.20
3	0.94	3.16	0.72	2.58	1.80	1.30	1.52	1.38
4	.....	2.50	0.58	2.12	0.46	1.24	0.02	1.78
5	.....	2.30	.....	2.36	.....	1.14	.....	1.16
Total....	22.16	15.14	20.48	13.38	17.94	6.90	19.10	8.96

perature. If the variations with temperature are large, the latter should be controlled; on the other hand, if the effect is insignificant, such precautions may not be necessary.

For this study single-base soils were electrodialyzed for 2 hours at different temperatures, a current of 0.1 ampere being used. The perforated copper funnel forming the anode was surrounded by a glass funnel around which water of the required temperature was circulated. The variations in temperature could be kept within 2°C. by this simple device; this was quite satisfactory for the purpose of this investigation. For higher temperatures a supplementary electric heater was inserted in the soil suspension. The results, plotted in figure 3, show that there is perhaps an optimum temperature, somewhere about 30°C., at which the rate of electrodialysis is the highest. This maximum is most prominent in the case of Ca-soil. It is difficult to find any valid cause for the maxima, especially as the magnitude of the difference does not follow any definite order, though the convexity occurs at virtually the same tem-

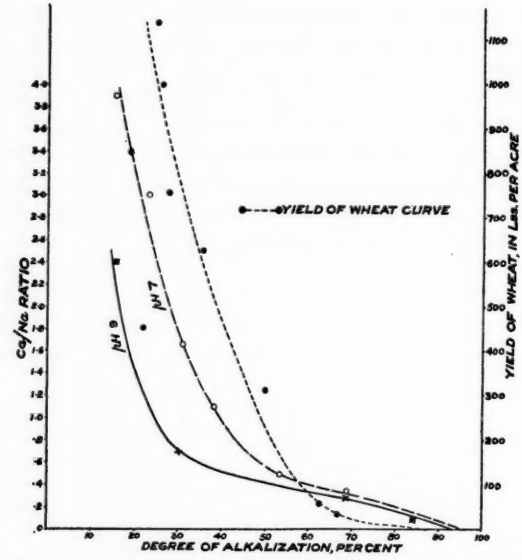


FIG. 2. RELATION BETWEEN DEGREE OF ALKALIZATION AND Ca/Na RATIO IN THE ELECTRODIALYZATE AND YIELD OF WHEAT

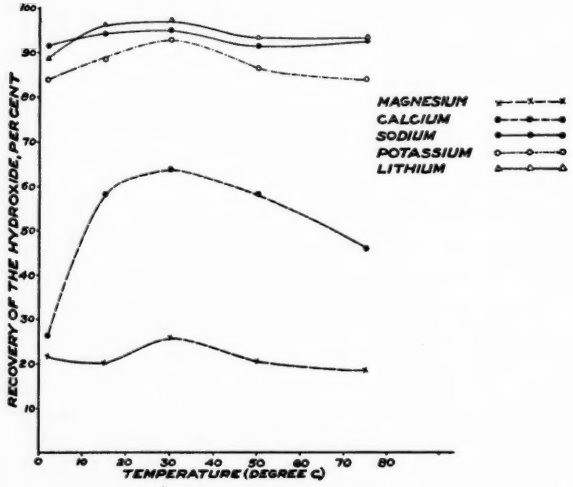


FIG. 3. EFFECT OF TEMPERATURE ON THE SPEED OF ELECTRODIALYSIS

perature in all cases. The results, however, are so regular that any possibility of side effects even at high temperatures seems to be ruled out. It is clear from these results that the effect of temperature on the rate of electrodialysis, on the whole, is so slight that no control of this factor appears necessary.

SUMMARY

Electrodialysis of soils at different pH values and varying degrees of alkalization has been studied. The rate of electrodialysis increases with the increase in pH value.

The variations in the ratio of Ca/Na in the electrodialyzate of alkali soils fit in with the hypothesis that the cause of infertility in such soils lies in the deficiency of available Ca.

The rate of electrodialysis reaches a maximum at about 30°C., but the effect of temperature, on the whole, is slight.

REFERENCES

(1) PURI, A. N. 1933 Relation between exchangeable sodium and crop yield in Punjab soils and a new method of characterizing alkali soils. Punjab Irrig. Res. Inst. Res. Pub. 4: 1–4.

(2) PURI, A. N. 1936 Estimating exchangeable calcium and other cations in soils. *Soil Sci.* 42: 47–59.

(3) PURI, A. N., AND HOON, R. C. 1937 Studies in electrodialysis of soils: I. Electrodialysis by the rotating electrode. *Soil Sci.* 43: 305–309.

(4) PURI, A. N., AND HOON, R. C. 1937 Studies in electrodialysis of soils: II. Polarographic current-voltage curves. *Soil Sci.* 44: 399–408.

(5) PURI, A. N., AND HOON, R. C. 1938 Studies in electrodialysis of soils: III. Speed of electrodialysis of various cations. *Soil Sci.* 45: 309–313.

AUTHOR INDEX

Albrecht, W. A., Graham, E. R., and Ferguson, C. E. Plant growth and the breakdown of inorganic soil colloids, 455–458.

Allen, E. K. *See* **Allen, O. N.**

Allen, O. N., and Allen, E. K. Root nodule bacteria of some tropical leguminous plants: II. 63–76.

Barnette, R. M. *See* **Rogers, L. H.,** Gall, O. E., and.

Blair, A. W., and Prince, A. L. Studies on nitrogen, phosphorus and mineral requirements of alfalfa, 459–466.

Bouyoucos, G. J. Effect of organic matter on water-holding capacity and wilting point of mineral soils, 377–383.

Boynton, D. Capillary tension as a measure of the pore space unoccupied by water in some dense orchard subsoils, 347–352.

Bracken, A. F. *See* **Greaves, J. E.**

Brown, S. M. *See* **Kelley, W. P.,** Woodford, A. O., Dore, W. H., and.

Burton, J. C., and Wilson, P. W. Host plant specificity among the Medicago in association with root-nodule bacteria, 293–303.

Coleman, O. H., and Gardner, R. Comparison of methods of determining small quantities of HCN, 409–413.

Cordon, T. C. *See* **Waksman, S. A.; Waksman, S. A.,** and Hulpoi, N.; **Waksman, S. A.,** Umbreit, W. W., and.

Cordon, T. C., and Haenseler, C. M. Bacterium antagonistic to *Rhizoctonia solani,* 207–215.

De, P. K. *See* **Starkey, R. L.**

Dhawan, C. L. *See* **Puri, A. N.,** Hoon, R. C., and.

Dore, W. H. *See* **Kelley, W. P.,** Woodford, A. O., and Brown, S. M.

Ferguson, C. E. *See* **Albrecht, W. A.,** Graham, E. R., and.

Fraps, G. S., and Sterges, A. J. Effect of phosphates on nitrifying capacity of soils, 115–121.

Gall, O. E. *See* **Rogers, L. H.,** and Barnette, R. M.

Gardner, R. *See* **Coleman, O. H.**

Gerdel, R. W. Preservation of small core soil samples, 353–355.

Gieseking, J. E. Mechanism of cation exchange in montmorillonite-beidellite-nontronite type of clay minerals, 1–13.

Godfrey, G. H. Control of nut grass with chloropicrin, 391–395.

Graham, E. R. *See* **Albrecht, W. A.,** and Ferguson, C. E.

Greaves, J. E., and Bracken, A. F. Influence of cropping on the nitrogen-fixing powers of soil, 201–206.

Haenseler, C. M. *See* **Cordon, T. C.**

Heyward, F. Moisture relationships of soils from burned and unburned longleaf pine forests, 313–327.

Hoon, R. C. *See* **Puri, A. N.; Puri, A. N.,** and Dhawan, C. L.

Hulpoi, N. *See* **Waksman, S. A.,** Cordon, T. C., and.

Jenny, H., and Overstreet, R. Cation interchange between plant roots and soil colloids, 257–272.

Kelley, W. P. Effect of dilution on water-soluble and exchangeable bases of alkali soils and its bearing on salt tolerance of plants, 367–375.

Kelley, W. P., Woodford, A. O., Dore, W. H., and Brown, S. M. Comparative study of the colloids of a Cecil and a Susquehanna soil profile, 175–193.

McKaig, N., Jr. *See* **Roller, E. M.**

Metzger, W. H. Nature, extent, and distribution of fertilizer residues in soil of some old fertility plats, 15–26.

Moxon, A. L. *See* **Olson, O. E.**

Nair, K. M. *See* **Singh, B. N.**

Olson, O. E., and Moxon, A. L. Availability, to crop plants, of different forms of selenium in the soil, 305–311.

Overstreet, R. *See* **Jenny, H.**

Prince, A. L. *See* **Blair, A. W.**

Puffeles, M. Effect of saline water on Mediterranean loess soils, 447–453.

Puri, A. N., and Hoon, R. C. Physical characteristics of soils: III, 415–423.

Puri, A. N., Hoon, R. C., and Dhawan, C. L. Studies in electrodialysis of soils: IV, 479–485.

Puri, A. N., and Puri, B. R. Physical characteristics of soils: II, 77–81.

Puri, A. N., and Uppal, H. L. Base exchange in soils: I, 245–253.

Puri, B. R. *See* **Puri, A. N.**

Ravikovitch, S. Influence of exchangeable cations on availability of phosphate in soils, 357–366.

Rogers, L. H., Gall, O. E., and Barnette, R. M. Zinc content of weeds and volunteer grasses and planted land covers, 237–243.

Roller, E. M., and McKaig, N., Jr. Critical studies of phenoldisulfonic acid method for determination of nitrates, 397–407.

Shive, J. W. *See* **Wadleigh, C. H.**

Singh, B. N., and Nair, K. M. Is sunlight a factor in nitrogen transformation in soils?, 285–291.

Singh, B. N., and Singh, L. B. Relative absorption of nutrients by weeds of arable land, 227–235.

Singh, L. B. *See* **Singh, B. N.**

Starkey, R. L., and De, P. K. New species of Azotobacter, 329–343.

Sterges, A. J. *See* **Fraps, G. S.**

Stevens, K. R. Chemical nature of organic matter in differently cropped arid soils, 27–31.

Toth, S. J. Stimulating effects of silicates on plant yields in relation to anion displacement, 123–141.

Turnage, W. V. Desert subsoil temperatures, 195–199.

Turk, L. M. Effect of mineral elements on microbiological activities in muck soils, 425–445.

Umbreit, W. W. *See* **Waksman, S. A.,** and Cordon, T. C.

Uppal, H. L. *See* **Puri, A. N.**

Wadleigh, C. H., and Shive, J. W. Microchemical study of effects of boron deficiency in cotton seedlings, 33–36; base content of corn plants as influenced by pH of substrate and form of nitrogen supply, 273–283.

Wadsworth, H. A. Factors influencing heat of wetting of soils, 385–390; rates of temperature changes in soils at various moisture contents, 467–471; effect of temperature upon moisture-content—surface-force curve of soil, 473–478.

Waksman, S. A., and Cordon, T. C. Thermophilic decomposition of plant residues in composts by pure and mixed cultures of microorganisms, 217–225.

Waksman, S. A., Cordon, T. C., and Hulpoi, N. Influence of temperature upon the microbiological population and decomposition processes in composts of stable manure, 83–113.

Waksman, S. A., Umbreit, W. W., and Cordon, T. C. Thermophilic actinomycetes and fungi in soils and in composts, 37–61.

Wall, M. E. Role of potassium in plants: I, 143–161.

Wilson, P. W. *See* **Burton, J. C.**

Woodford, A. O. *See* **Kelley, W. P.,** Dore, W. H., and Brown, S. M.

SUBJECT INDEX

Actinomycetes—
  in manure composts, 94
  in soils, 37
  thermophilic, 48
Alfalfa, mineral requirements, of, 459–466
Alkali soils, a discussion in relation to base exchange, 367
Alkalization of soil affecting electrodialysis, 479
Aluminum sulfate, effect on muck soils, 431
Ammonia—
  accumulation in muck soils, 428
  exchangeable, effect on liberation of phosphate, 360
  for plants, 277
  nitrogen in boron deficient plants, 35
Aniline in exchange reactions, 5, 7
Anion displacement, 123
Azotobacter, a new species, 329–338
Bacteria—
  cowpea group of root-nodule, 63
  in manure composts, 94
  in soils, 41, 441
  numbers as affected by dispersion, 442
  Rhizoctonia solani, a bacterium antagonistic to, 207–214
  root-nodule—
    of tropical legumes, 63–76
    studies, 293
Barium chloride, effect on muck soils, 431
Base—
  content of corn plants, 273–283
  exchange—
    a discussion, 367
    capacity as affected by fertilizers, 21
    in soils, methods, 245–253
  recovery upon electrodialysis, 483
Beidellite—
  distribution in soil horizons, 181
  montmorillonite-nontronite type of clay minerals, 1
Books, *see* Book reviews at end of letter B.
Boron—
  deficiency, 33
  effect on muck soils, 431
Brucine in exchange reactions, 5

BOOK REVIEWS

Algae, 169
Chemistry of the Amino Acids and Proteins, 168
Collings, G. H. Commercial Fertilizers. Second edition, 168
Ferns of the Southeastern States, 165
Fertilizers, Commercial, 168
Genetics, 171
Humus, 255
Jasny, N. Research Methods on Farm Use of Tractors, 164
Kubiëna, W. L. Micropedology, 163
Micropedology, 163
Ogg, W. G. (editor) Macaulay Institute for Soil Research. Collected Papers, v. 1, 166
Rothamsted Experimental Station Report for 1937, 167
Schmidt, C. L. A. Chemistry of the Amino Acids and Proteins, 168
de'Sigmond, A. A. J. Principles of Soil Science, 170
Small, J. K. Ferns of the Southeastern States, 165
Snedecor, G. W. Statistical Methods. Second edition, 163
Soil, Humus, 255
Soil, Micropedology, 163
Soil Research, Collected Papers, Macaulay Institute for, 166
Soil Science, Principles of, 170
Statistical Methods, 163
Tiffany, L. H. Algae. The Grass of Many Waters, 169
Tractors, Research Methods on Farm Use of, 164
Waksman, S. A. Humus—Origin, Chemical Composition, and Importance in Nature. Second edition, 255
Walter, H. E. Genetics. An Introduction to the Study of Heredity. Fourth edition, 171

Calcium—
  exchangeable, effect on phosphate liberation, 360
  in alfalfa, 462
  saturated soil, availability of phosphate in, 359
  weeds rich in, 232
Capillary tension as a measure of pore space, 347
Carbohydrate metabolism, 157
Carbon dioxide—
  effect on release of elements, 457
  from mucks, 439

Cation—*see* also Base
exchange mechanism, 1–13
exchangeable, effect on phosphate availability, 357
interchange between roots and soil colloids, 237–272
preparation of soil for, study, 357
Chloropircrin, control of nut grass with, 391
Clay—
liberation of nutrients from, 457
minerals, cation exchange in the montmorillonite-beidellite-nontronite, 1
Colloids—
cation interchange between plant roots and, 257
dehydration, 179
molecular ratios in, 177
optical properties, 183
plant growth and breakdown of inorganic soil, 455–458
profile study of soil, 175
X-ray analyses, 185
Composts, actinomycetes and fungi in, 37
Copper sulfate, effect on muck, 429
Corn, base content, 273
Cotton seedlings, effect of boron deficiency, 33
Crops—
phosphorus in, 134
silica in, 134
Electrodialysis of soils, 479
Fertilizer—
effect on exchange capacity, 21
residues, distribution in the soil, 15–26
Forests—
burned and unburned, 318
pine, moisture in, 313
Fungi—
in manure composts, 94
in soils, 37, 441
Gelatin in exchange reactions, 6
Halloysite type of colloid, 190
Heat of wetting of soils, 385, 415
Hydrocyanic acid, methods of determining, 409–413
Hydrogen-ion concentration (pH)—
effect on electrodialysis of soils, 479
influence on base content of corn plants, 273
ultimate, as affected by fertilizers, 21
Iodide, tributyl mono-heptyl ammonium, in exchange reactions, 5
Irrigation water problem, 448
Kaolinite type of colloids, 190
Lime—
comparison with silicates of calcium and magnesium, 131
effect on muck soils, 434
Lipman, Jacob Goodale, obituary note, facing 346
Magdala red in exchange reactions, 6
Magnesium—
exchangeable, effect on phosphate liberation, 360
in alfalfa, 462
Manganese sulfate, effect on muck soils, 430
Manure—
effect on phosphorus distribution in soils, 19
horse—
composition, 87
microbes in, 43
infusion, effect on muck soils, 435
stable, decomposition, 47, 83
Medicago sativa, ability to fix nitrogen, 301
Methylene blue in exchange reactions, 4
Microbiological process in muck soils, 425
Microorganisms—*see* also Bacteria, Fungi, Actinomycetes
temperature, effect on, in stable manure composts, 83–98
thermophilic decomposition of plant residues by, 217–224
Minerals—
for alfalfa, 459
metabolism by tomatoes, 143
Moisture—
contents, temperature changes in soils affecting, 467, 473
field capacity, measure, 350
in pine forests, 313
Montmorillonite—
beidellite-nontronite type of clay minerals, 1
distribution in soil horizons, 181
Muck soils, microbiological activities in, 425
β-Naphthylamine in exchange reactions, 5, 7, 8
Nitrate—
accumulation in muck soils, 428
and ammonia for plants, 277
nitrogen for plants, 274
phenoldisulfonic acid method of determining, 397
Nitrification—
phosphate effect on, 115
studies, 437

Nitrogen—
changes in horse manure, 91
fixing power of soils, 201
Medicago sativa, ability to fix, 301
metabolism by tomatoes, 143, 154
nitrate in boron deficient plants, 35
removal by alfalfa, 463
requirements of alfalfa, 459
sunlight as a factor in, transformation in soil, 285
supply, effect on base content of corn, 273
weeds rich in, 232
Nontronite-beidellite-montmorillonite type of clay minerals, 1
Nut grass, control with chloropicrin, 391
Nutrients, adsorption by weeds, 227
Orchard subsoils, pore space in, 347
Organic matter—
effect on—
actinomycetes, 42
water holding capacity of soils, 377–383
in arid soils, 27
Phosphate—
availability, 357
displacement, 127
Phosphorus—
available, and soil treatment, 465
distribution in soil, 16, 18
effect on nitrifying capacity of soils, 115–121
in alfalfa, 461
in sudan grass, 134
Potassium—
deficiency symptoms, 152
exchangeable, effect on phosphate liberation, 360
fixation, 24
in alfalfa, 461
role in plants, 143–161
weeds rich in, 232
Pyridine in exchange reactions, 4
Roots, contact between, and soils, 259
Rossi, Giacomo, obituary note, 173
Saline water, effect on loess soils, 447
Selenium, availability to crops, 305–311
Shorey, Edmund Cecil, obituary note, 344
Silica—
in sudan grass, 134
sesquioxide ratio in soil colloids, 177
Silicates—
adsorption, 129
effect on plant yields, 123–140
Sodium—
chloride, effect on muck soils, 429
exchangeable, effect on phosphate liberation, 360
nitrate, effect on movement of phosphorus, 19, 23
sulfate, effect on muck soils, 431
Soil—
capillary tension, as a measure of pore space, 347
colloids, *see* Colloids
desert, temperature, 195–199
fertility plots, 15
fertilizer residues in, 15
horizons, colloids in, 181
moisture determination, 473
nitrogen fixing power, 201–206
nitrogen transformation by sunlight, 285–291
phosphate availability in, 357–366, 465
pore space, 347
profile, colloids in, 175–193
samples, preservation, 353
selenium in, 305
series, analyses, descriptions, or experiments with—
Bell, 116; Cecil, 175; Cisne, 2; Colts Neck, 123, 129; Crockett, 116, 117; Duval, 116; Falls, 117; Hartsburg, 2; Houston, 116, 117; Leaf, 116; Lufkin, 116; Miles, 116, 117; Norfolk, 117; Pryor, 116; Putnam, 2; Quanah, 116; Refugio, 117; Ruston, 116; Sumter, 116, 117; Susquehanna, 117, 175; Tabor, 116; Vernon, 117; Victoria, 117; Webb, 116; Wilson, 116
surface—force curve, as affected by moisture, 473
Soils—
actinomycetes, 37
alkali, a discussion of, in relation to base exchange, 367
base exchange, *see* Base exchange
calcium-saturated, 359
contact between, and roots, 259
electrodialysis, 479–485
fungi, 37
heat of wetting, 385–390
hydrogen-saturated, 359
loess, of the Mediterranean, 447–453
mechanical analyses, 77
moisture relationships in forests, 313–324

Soils—(*continued*)
muck, microbiological activities in, 425
nitrifying capacity, 115, 357
organic matter in arid, 27–31
Palestine, analyses, 450
phosphates in, 115
physical characteristics, 77–81, 415–423, 449
sub—
desert, temperatures, 195
pore space in, 347
temperature changes in, 467
wilting point, 377
Sulfur, effect on muck soils, 431
Temperature, effect on—
electrodialysis, 479
Temperature, effect on—(*continued*)
microbial population, 83
moisture in soil, 467, 473
Tomatoes, metabolism, 143
Water—
analyses, 448
saline, effect on loess soils, 447
Weeds—
adsorption of nutrients by, 227–235
zinc content, 237
Wilting point of mineral soils, 377
X-ray analyses of soil colloids, 185
Zinc—
content of weeds and other plants, 237–243
sulfate, effect on muck soils, 431

Science
Library

JUN 15 1939

THIS NUMBER COMPLETES VOLUME 47

Volume 47 June, 1939 Number 6

SOIL SCIENCE

174

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

Contents

Effect of Certain Mineral Elements on Some Microbiological Activities in Muck Soils. L. M. Turk 425

Effect of Saline Water on Mediterranean Loess Soils. M. Puffeles ... 447

Plant Growth and the Breakdown of Inorganic Soil Colloids. W. A. Albrecht, Ellis R. Graham, and Carl E. Ferguson 455

Studies on the Nitrogen, Phosphorus, and Mineral Requirements of Alfalfa. A. W. Blair and A. L. Prince 459

Rates of Temperature Changes in Soils at Various Moisture Contents. H. A. Wadsworth 467

Effect of Temperature Upon the Moisture-Content—Surface-Force Curve of Soil. H. A. Wadsworth 473

Studies in Electrodialysis of Soils: IV. Effect of Temperature, pH Value, and Degree of Alkalization. Amar Nath Puri, R. C. Hoon, and C. L. Dhawan 479

Index 487

Published Monthly by THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MARYLAND, U. S. A.

Copyright, 1939, The Williams & Wilkins Company

Made in United States of America

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

ADVISORY STAFF

A. W. Blair, J. S. Joffe, L. L. Lee, H. C. McLean, A. L. Prince, J. W. Shive, H. B. Sprague, R. L. Starkey, and S. A. Waksman, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

CONSULTING EDITORS

F. J. ALWAY
University of Minnesota, St. Paul, Minn.

K. ASO
Imperial University, Tokyo, Japan

C. BARTHEL
Lantbrukshögskolan, Uppsala, Sweden

RICHARD BRADFIELD
College of Agriculture, Cornell University, Ithaca, New York

B. C. BURT
Imperial Council of Agricultural Research, New Delhi, India

H. J. CONN
New York State Experiment Station, Geneva, N. Y.

ALBERT DEMOLON
Ministry of Agriculture, Paris, France

E. B. FRED
University of Wisconsin, Madison, Wis.

V. V. GEMMERLING
Agronomitseskii Institute, Moskovskii Universitat, Moscow, U. S. S. R.

J. E. GREAVES
Utah Agricultural College, Logan, Utah

D. J. HISSINK
Soil Science Institute, Groningen, Holland

D. R. HOAGLAND
University of California, Berkeley, California

O. LEMMERMANN
Berlin-Zehlendorf, Germany

C. B. LIPMAN
University of California, Berkeley, Calif

BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md

HENRIK LUNDEGÅRDH
Centralanstalten för jordbruksförsök, Stockholm, Sweden

M. M. MCCOOL
Boyce-Thompson Institute, Yonkers, N. Y.

W. H. MACINTIRE
University of Tennessee, Knoxville, Tenn.

S. MATTSON
Lantbrukshögskolan, Uppsala, Sweden

S. MIKLASEWSKI
Institute of Soil Science, Warsaw, Poland

E. A. MITSCHERLICH
University of Königsberg, Germany

C. A. MOOERS
University of Tennessee, Knoxville, Tenn.

D. N. PRIANISHNIKOV
Timiryazev Academy, Moscow, U. S. S. R.

THEO. REMY
Bonn-Rhein, Germany

G. W. ROBINSON
University College of North Wales, Bangor, Wales

E. J. RUSSELL
Rothamsted Experimental Station, Harpenden, England

O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.

ALEXIUS A. J. DE'SIGMOND
Royal Hungarian Joseph University of Technical Sciences, Budapest, Hungary

N. M. TULAIKOV
Agricultural Experiment Station, Saratov, U. S. S. R.

EMILE H. DEL VILLAR
Forestry Institute, Madrid, Spain

S. WINOGRADSKY
Pasteur Institute, Paris, France

Entered as second-class matter, May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879.
Subscription price $5.00 per volume. Single numbers, $1.00

SOIL SCIENCE

Manuscripts submitted for publication and communications concerning editorial matters should be sent to *Soil Science*, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Twenty-five reprints, without covers of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

SOIL SCIENCE is issued monthly. Each issue consists of approximately 85 pages at present. Two volumes a year are issued at present. Each volume consists of approximately 500 pages.

Subscription price: $5.00 per volume in the United States and countries within the Postal Union. $5.50 outside the Postal Union.

Claims for copies lost in the mails must be received within 30 days (domestic), and 90 days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

New subscription and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro-rata value of such numbers will be credited to the next volume, and the renewal invoice therefore adjusted accordingly.

Subscriptions should be renewed promptly—To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, should be sent to the Publishers or to Agents listed below.

Index for Volumes I-XXV is available at $3.00 so long as present supply lasts.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Angus & Robertson, Limited, 89–95 Castlereagh Street, Sydney.

For Belgium: Henri Lamertin, 58 Rue Coudenberg, Bruxelles.

For the British Empire, except Australia and Canada: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, WC. 2, London, England.

For Canada: Wm. Dawson & Sons, Ltd., 91 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann Co., Inc., Berlin-Charlottenburg 4, Bismarckstrasse 40.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 6 Nihonbashi Tori-Nichome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

PUBLISHERS OF: *Medicine, Journal of Urology, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Journal of Industrial Hygiene and Toxicology, Quarterly Review of Biology, Journal of Bacteriology, Journal of Organic Chemistry, Annals of the Pickett-Thomson Research Laboratory, Chemical Reviews, Soil Science, Social Forces, Journal of Comparative Psychology, Mental Measurement Monographs, Occupational Therapy and Rehabilitation, The American Journal of Clinical Pathology, Journal of Physical Chemistry, Philosophy of Science, Medico-Legal and Criminological Review, Medical Classics, Journal of Investigative Dermatology.*

NUTRIENT SOLUTIONS

THE excellent results obtained at the New York State Agricultural Station from an application of nutrients in solution to tomatoes at time of transplanting in a field already well fertilized is quickening the interest in nutrient solutions. Along with other important research on methods of application, undoubtedly more attention will be turned to the increased efficiency of fertilizer applied in solution and at times when the plants can use it before it is fixed by the soil or leached from it. In this work the possibility of making available to plants all of the potash necessary for maximum growth and quality is seen.

AMERICAN POTASH INSTITUTE, INC.

INVESTMENT BUILDING • • • WASHINGTON, D. C.

Branch Offices: Beans Bldg., San Jose, Calif. Life Building, Lafayette, Ind.
Mortgage Guarantee Bldg., Atlanta, Ga. Yates Building, Hamilton, Ont.

Second Edition

SELMAN A. WAKSMAN:

Professor of Soil Microbiology, Rutgers University, and Microbiologist, New Jersey Agricultural Experiment Station.

HUMUS

Origin, Chemical Composition and Importance in Nature

521 pages
44 illustrations
$6.50

THE first edition has sold out in 2½ years, a much shorter period than could be expected. However, the rapidly accumulating information on the subject of humus and the growing recognition of the importance of humus in soil fertility and plant growth has necessitated considerable revision. The book retains of course its orginality of approach and the broad appreciation of the many aspects of the subject.

● The work is a mine of information, and it is difficult to see how any worker in soil science or related subjects can afford to be without it.—*Journal of Association of Official Agricultural Chemists.*

● Le mérite de S. Waksman est d'autant plus grand que dans aucun domaine de la science du sol n'a régné jusqu'ici une confusion aussi complète.—*Annales Agronomiques.*

● In reading the book through one is impressed by the writer's thorough grasp of his subject and by the fact that it is written by a man who is himself a laboratory man yet au fait with field conditions, and a scientific producer of new thought and new knowledge.—*Scientific Agriculture.*

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves. Baltimore, Maryland